JOURNAL OF AGRICULTURAL RESEARCH

VOL. 62 WASHINGTON, D. C., MARCH 15, 1941 No. 6.

EFFECT OF COOL STORAGE OF EASTER LILY BULBS ON SUBSEQUENT FORCING PERFORMANCE [1]

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INTRODUCTION

Easter lily bulbs produced in the Southern States usually are ready to dig before the florist is ready to receive them for forcing. Some storage period is therefore necessary. The present study conducted at the United States Horticultural Station, Beltsville, Md., was begun in the fall of 1935 with the purpose of finding a storage practice that would maintain the forcing quality of the bulbs and reduce to a minimum the occurrence of rot, shriveling, and premature sprouting. Short exposures to cool temperature kept the bulbs in good condition and showed a marked effect in accelerating bloom. This latter effect was examined in further detail, and the present paper summarizes 3 years' trials showing that specific preplanting storage treatments are a convenient means of controlling the time of flowering in Easter lilies.

MATERIAL AND METHODS

The horticultural varieties of *Lilium longiflorum* Thunb. included in these studies and the sources of the experimental material were Creole from Louisiana, Croft from Oregon, Erabu and Giganteum from Japan, and Harrisii from Bermuda. The name "Croft" has been applied for convenience to a commercial type grown in Oregon without varietal designation and purchased direct from growers each year. One of the 1936–37 stocks of Creole and the 1937–38 stock of Harrisii were also bought direct from growers. All others were purchased through dealers. Attempts were made to obtain each stock promptly after digging to avoid the complication of undesired storage practices before the bulbs were received for the experiments. In general these attempts were not successful, and the resulting discrepancies will be discussed herein.

Bulbs 7 to 9 inches in circumference were specified in all purchases. The mean circumference was determined from 50 random bulbs of each variety and ranged from 7.20 to 8.19 inches, with the exception of one Creole lot (C_1 37 in table 2) in which the circumference averaged only 5.57 inches. The last-named lot was supplied after standard sizes had been sold. The *Lilium longiflorum* seedlings included in one trial were random samples of open-pollinated progenies of the Croft variety. These had been grown in the greenhouse approximately 1 year from seed when the trials were begun.

[1] Received for publication November 25, 1940.

The number of bulbs per unit trial was 25, but if one or more failed to grow or to flower, the means were computed for the number flowering. This was considered sound practice, since the factors responsible for failure to grow or to bloom were apparently independent of the factors under test. Two packing materials, moistened peat moss and dry sand (or soil), were used. Throughout the 1935–36 and 1936–37 studies the individual test lots were repacked for storage tests in peat moss which had been thoroughly wet and from which the excess water was pressed out with the hands. In 1937–38 this moist-peat pack was compared with the original dry sand (Bermuda) or sandy loam (Louisiana) in which the bulbs were shipped.

Storage facilities were provided by the storage and transportation project of this Division at the Arlington Experiment Farm, Arlington, Va. Temperatures were controlled within a range of approximately ±1° F. from the values stated, and relative humidity was maintained at a level of approximately 80 percent. Bulbs subjected to coldframe treatment were potted as for greenhouse planting and plunged in an open unheated frame, where they were exposed to the outdoor temperatures of Beltsville, Md., for the stated intervals. At the close of these intervals the pots were moved to the greenhouse.

In the greenhouse the bulbs were grown singly in 6-inch clay pots in a composted soil with which a liberal amount of bonemeal had been well mixed. The soil for each year's series of trials was prepared and mixed in advance. All trials were conducted in the same section of a greenhouse, the temperature of which was manually controlled, as far as practicable, to 65° F. in the daytime and 60° at night. These temperatures, however, were often far exceeded during the summer months.

1935–36 EXPERIMENT

The data for 3 varieties included in the first year's trials (1935-36) are shown in table 1. Since these varieties were received at different dates, the storage exposures and corresponding control plantings were made independently for each variety. It is apparent from table 1 that cool storage accelerates blooming, the difference in number of days to bloom between stored and control lots always proving highly significant. The height of flowers above the soil line also shows significant decreases in the stored lots as compared with the nonstored lots in the Croft variety. The number of flowers per bulb is decreased in stored lots of Croft and Erabu but not in Creole. The actual date of bloom is shifted less strikingly by storage treatment than is the number of days to bloom, since the stored lots were planted (and the coldframe lots moved to the greenhouse) 37 to 41 days later than the control lots. Data in table 1 from unselected seedlings exposed for two different intervals in the coldframe and those without such exposure to a cool period show similar trends.

Although the general trends toward earlier blooming, shorter stems, and fewer flowers were similar in the three varieties and in one group of seedlings tested, these responses were most striking in the Croft variety. In actual flowering dates the stored lots of Croft were far in advance of the controls, while the stored lots of Erabu bloomed slightly later but within 6 days of the controls. Accordingly the 1936–37 trials were planned to compare the response of different Easter lily varieties to similar storage treatments.

TABLE 1.—*Effect of storage treatment on time of blooming and quality of flowers of 4 varieties of Easter lily in 1935–36*

Variety and source	Circumference of bulbs on arrival [1]	Preplanting treatment	Date planted in greenhouse	Date of blooming	Days to blooming [1] [2]	Height [1]	Flowers per bulb [1]
	Inches		*1935*	*1936*	*Number*	*Inches*	*Number*
Croft, from Oregon.	7.92±0.07	None	Oct. 8	June 2	238.3±1.98	20.0±1.00	7.4±0.37
		Coldframe 41 days	Nov. 15	May 21	188.3±1.26	13.6± .35	6.4± .21
		Stored at 50° F. 41 days.	...do	Apr. 26	162.9± .82	11.6± .31	3.4± .14
		Stored at 36° F. 41 days.	...do	Apr. 21	158.2± .99	13.3± .33	3.4± .13
Creole, from Louisiana.	7.49± .06	None	Oct. 25	Apr. 26	184.4±1.10	26.3± .38	4.3± .16
		Coldframe 40 days	Dec. 4	Apr. 17	135.6± .56	24.0± .44	4.2± .16
		Stored at 50° F. 40 days.	...do	Apr. 21	139.0±1.08	23.8± .40	3.7± .24
		Stored at 36° F. 40 days.	...do	Apr. 24	142.6± .74	23.0± .47	4.2± .16
Erabu, from Japan.	7.87± .10	None	Nov. 12	...do	164.5±1.44	19.6± .80	7.2± .42
		Coldframe 37 days	Dec. 19	Apr. 25	128.0±1.01	17.2± .81	5.6± .40
		Stored at 50° F. 37 days.	...do	Apr. 28	131.8±1.16	20.7± .67	4.7± .43
		Stored at 36° F. 37 days.	...do	Apr. 30	133.2± .94	18.8± .82	5.3± .26
L. longiflorum (seedling).		None	Jan. 8 [3]	June 3	[4]144.7±3.43	23.6±1.35	5.4± .29
			1936				
		Coldframe 81 days	Jan. 10	May 5	116.6± .74	17.8± .56	6.0± .44
		Coldframe 182 days	Apr. 20	June 13	54.1± .76	15.6± .49	2.6± .29

[1] Mean and standard error.
[2] From date of planting in greenhouse.
[3] Date on which seed was planted.
[4] Computed as days after Jan. 10, 1936.

1936–37 EXPERIMENT

In the experiment of 1936–37 the varieties Giganteum and Harrisii were added to those previously tested. Two lots of Erabu, designated by the dealer as "Black Stem Regular" ($E_2$37 in table 2) and "Improved Early Flowering" ($E_1$37 in table 2), were included. The dealer did not state whether the early type was genetically or physiologically early, but the results obtained by the writer suggest that its difference from the Black Stem Regular is attributable to handling rather than to selection. Two stocks of Creole were used; one of regular commercial size obtained from a dealer after considerable delay, and one of smaller bulbs obtained direct from the producers. The Croft variety was delayed in arrival because of forest fires in Oregon.

Since it was planned to start storage trials on all varieties simultaneously to minimize differences in the conditions encountered during forcing, the early arrivals were held in their original packing until the later arrivals were received. The prolonged delay of Croft and Creole ($C_2$37) introduced an error that had not been anticipated. The early-arriving varieties stood for 5 to 7 weeks in the original cases in a room at 50° F., which constituted a cool storage treatment in the controls. The effect of this storage is shown in differences that appear between lots 1A, planted on arrival, and lots 1, planted November 2, after all lots (except Croft) were on hand. The duration of this unplanned storage interval may be computed from the differences between planting dates of lots 1A and 1 in table 2. Where no lot 1A appears, the variety was received just in time for the tests proper.

TABLE 2.—*Effect of storage treatment on time of blooming and quality of flowers in 1936–37*

CREOLE $C_1$37 (MEAN CIRCUMFERENCE OF BULBS, 5.57±0.5 INCHES)

Lot No.	Storage treatment: Temperature	Storage treatment: Interval	Date planted in greenhouse	Average date of blooming	Days to blooming [1 2]	Days to emergence [1 2]	Height [2]	Flowers per plant [2]	Size of flower [2] (length by diameter)
	°F.	Weeks	1936	1937			Inches	Number	Index number
1A	[3]	------	Sept. 12	Apr. 14	214.0±0.70	69.1±0.80	27.1±0.57	3.5±0.21	28.5±0.47
1	[4]	------	Nov. 2	Apr. 10	158.7± .58	35.7± .91	31.1± .53	3.0± .18	29.1± .38
2	50	5	Dec. 7	Apr. 12	125.8± .90	18.1±1.36	30.7± .89	2.7± .53	29.1± .60
3	40	5	...do.....	Apr. 16	129.8± .68	28.7± .90	29.4± .49	3.1± .10	28.8± .41
4	32	5	...do.....	Apr. 22	135.6± .82	34.2± .73	27.8± .47	3.4± .16	29.6± .51
9	[5]	5	...do.....	Apr. 18	131.9± .70	26.2± .75	27.4± .64	3.4± .15	29.8± .59
			1937						
6	50	10	Jan. 11	Apr. 22	101.0± .97	.6± .56	24.4± .49	1.3± .11	30.1± .69
7	40	10	...do.....	May 6	115.0±1.36	28.3±1.68	18.3± .74	2.4± .54	27.6± .66
10	32	17	Mar. 5	May 23	78.6±1.05	17.9±1.00	13.9± .55	2.4± .39	25.1± .46

CREOLE $C_2$37 (MEAN CIRCUMFERENCE OF BULBS, 7.55±0.15 INCHES)

			1936						
1	[4]	------	Nov. 2	Mar. 16	134.4±1.62	22.4±1.02	32.3±1.30	3.7±0.56	28.9±0.33
2	50	5	Dec. 7	Apr. 6	120.2±1.13	20.0±1.24	31.4± .90	2.9± .34	27.9± .47
3	40	5	...do.....	Apr. 14	128.0±1.31	28.7±1.41	30.9± .82	4.0± .43	28.5± .56
4	32	5	...do.....	Apr. 16	130.5±1.25	29.0± .81	33.1± .71	4.0± .39	28.1± .72
9	[5]	5	...do.....	Apr. 10	124.2±1.60	19.5±1.23	29.9±1.04	4.4± .48	28.1±1.22
			1937						
6	50	10	Jan. 11	Apr. 27	105.6±1.52	5.6±1.53	22.7± .87	1.7± .20	29.5± .66
7	40	10	...do.....	May 9	117.8±1.67	28.1±1.79	18.1±1.01	3.2± .39	27.5± .68

CROFT CT 37 (MEAN CIRCUMFERENCE OF BULBS, 7.28±0.08 INCHES)

			1936						
1	[4]	------	Nov. 5	Apr. 22	167.9±4.18	35.7±1.02	14.0±0.65	5.8±0.35	29.4±0.89
2	50	5	Dec. 7	Apr. 29	142.6±2.37	25.4±1.35	13.1± .31	5.8± .20	29.6± .89
3	40	5	...do.....	May 2	145.5±2.29	35.8±1.73	13.9± .65	4.6± .28	30.9± .72
4	32	5	...do.....	Apr. 30	143.9±1.84	37.0±1.26	11.2± .51	5.0± .33	29.2± .68
9	[5]	5	...do.....	May 2	145.8±1.71	33.6±1.11	11.3± .53	4.5± .29	29.8± .54
			1937						
6	50	10	Jan. 11	May 12	121.4±1.75	15.6±2.62	10.9± .38	3.2± .28	30.4± .71
7	40	10	...do.....	May 13	122.3±1.21	30.2± .92	10.3± .21	4.7± .35	28.2±1.93
10	32	17	Mar. 5	May 24	79.8± .85	17.4± .47	12.5± .36	5.4± .58	28.0± .53

EARLY ERABU $E_1$37 (MEAN CIRCUMFERENCE OF BULBS, 8.19±0.11 INCHES)

			1936						
1A	[3]	------	Sept. 26	Feb. 24	150.7±6.39	47.2±3.55	20.5±1.12	3.4±0.23	23.5±1.08
1	[4]	------	Nov. 2	Mar. 12	129.5±2.44	28.4± .84	25.9± .87	4.5± .33	24.5± .67
2	50	5	Dec. 7	Mar. 30	113.4±1.12	12.8± .88	26.5±1.15	4.2± .27	23.0± .50
3	40	5	...do.....	Apr. 8	122.1±2.10	26.5±1.16	20.0± .85	2.8± .72	23.2± .90
4	32	5	...do.....	Apr. 11	124.8±1.67	30.5±1.09	22.8±1.07	4.0± .34	23.8±1.76
9	[5]	5	...do.....	Apr. 4	118.5±1.56	19.9± .77	23.7±1.32	4.7± .35	24.2± .94
			1937						
6	50	10	Jan. 11	Apr. 21	99.6±1.32	9.7±2.46	14.2± .63	2.5± .23	22.2±.79
7	40	10	...do.....	Apr. 30	109.2±1.67	18.8±1.48	15.7± .75	2.4± .13	22.3±.82

ERABU $E_2$37 (MEAN CIRCUMFERENCE OF BULBS, 7.64±0.10 INCHES)

			1936						
1A	[3]	------	Sept. 26	Mar. 14	169.0±2.47	45.3±1.80	20.6±0.92	3.7±0.41	21.6±0.63
1	[4]	------	Nov. 2	Apr. 1	149.5±1.75	34.2±1.65	28.0± .94	5.4± .41	23.5± .75
2	50	5	Dec. 7	Apr. 12	125.7±1.48	16.1± .79	28.1± .82	4.6± .34	24.2± .74
3	40	5	...do.....	Apr. 18	132.1±1.20	26.8± .87	25.1± .84	4.4± .26	22.9± .60
4	32	5	...do.....	Apr. 19	133.3±1.48	30.6± .78	21.6± .81	5.7± .33	22.6± .56
9	[5]	5	...do.....	Apr. 24	138.5±1.58	27.4± .87	23.3± .99	5.1± .33	24.2± .61
			1937						
6	50	10	Jan. 11	May 5	114.4±2.23	10.4±2.41	18.1± .87	2.9± .25	22.3±1.11
7	40	10	...do.....	May 9	117.9±1.50	25.2±1.39	16.2± .85	3.0± .78	22.6± .53
10	32	17	Mar. 5	June 1	88.3±1.67	25.5±1.55	19.8± .70	3.1± .53	22.9± .91

Footnotes at end of table.

TABLE 2.—*Effect of storage treatment on time of blooming and quality of flowers in 1936–37*—Continued

GIGANTEUM (MEAN CIRCUMFERENCE OF BULBS, 7.20±0.07 inches)

Lot No.	Storage treatment: Temperature	Storage treatment: Interval	Date planted in greenhouse	Average date of blooming	Days to blooming	Days to emergence [1 3]	Height	Flowers per plant [2]	Size of flower (length by diameter)
	°F.	Weeks	*1936*	*1937*			*Inches*	*Number*	*Index number*
1A	(3)	-------	Oct. 20	May 5	197.0±1.00	33.5±0.73	15.2±0.55	5.5±0.21	24.4±0.43
1	(4)	-------	Nov. 2	May 4	182.6± .96	37.1±1.09	14.0± .56	5.8± .43	24.1± .84
2	50	5	Dec. 7	do	148.1±1.32	20.3±1.11	13.7± .57	4.7± .34	24.4± .56
3	40	5	do	May 9	153.0±1.28	33.1±1.20	12.1± .48	4.4± .23	24.2± .41
4	32	5	do	May 11	155.2±1.44	36.4±1.44	9.0± .44	5.0± .32	22.2± .66
9	(5)	5	do	May 4	148.2± .99	31.3±1.77	10.1± .41	5.0± .29	24.2± .61
			1937						
6	50	10	Jan. 11	May 14	122.8±1.40	11.0±3.16	9.1± .46	2.6± .22	23.5± .98
7	40	10	do	do	122.8± .96	29.7±2.74	9.3± .35	3.1± .19	24.5± .48
10	32	17	Mar. 5	June 2	89.2±2.13	24.6±1.34	13.2± .96	2.7± .22	24.3±2.34

HARRISII (MEAN CIRCUMFERENCE OF BULBS, 7.97±0.10 inches)

			1936						
1A	(3)	-------	Sept. 26	May 1	217.3±2.33	86.0±2.21	20.5±0.90	2.7±0.30	30.7±2.18
1	(4)	-------	Nov. 2	May 3	181.8±3.23	55.2±3.72	21.6± .78	3.2± .25	32.0±1.46
2	50	5	Dec. 7	May 16	160.2±2.80	40.7±2.54	18.5± .90	2.7± .24	31.6± .85
3	40	5	do	May 14	157.6±3.04	39.9±1.07	18.6± .82	2.9 ±.25	34.0± .58
			1937						
6	50	10	Jan. 11	May 24	133.4±3.56	25.6±5.57	16.8± .84	2.5± .23	32.5± .85
7	40	10	do	May 28	137.2±2.51	33.2±2.51	17.5± .89	2.9± .16	33.2± .90

[1] From date of planting in greenhouse.
[2] Mean and standard error.
[3] Bulbs planted in greenhouse on arrival.
[4] Bulbs planted in greenhouse Nov. 2 (except Croft).
[5] Unheated coldframe.

The storage temperatures and intervals and the forcing data are detailed in table 2. An index of the size of flower, that is, the product of the length by the diameter of the flower when approximately prime, is also presented in table 2. This index is believed to be a more reliable measure than length or diameter alone, since these measures change as the flower opens and fades but tend to equalize each other in the index. The difference between any two means is treated as significant (5-percent level) when found to be twice its standard error.

Bulbs kept in cool storage bloomed in a shorter time from date of planting than the controls in all cases, the differences proving highly significant with rare exceptions ($C_2$37, lot 4). However, the actual dates of blooming were never significantly earlier than those of the untreated lots. The maximum effects of storage treatments appear in Creole $C_1$37, the stock shipped to Beltsville direct from Louisiana. Significant shortening of the number of days required for flowering of stored lots appeared in all the varieties tested, indicating that the accelerating effect of low temperature is not peculiar to any one type of Easter lily. The degree of acceleration of flowering brought about by cool storage again varied among varieties, but the variety most influenced by treatment in 1935–36 (Croft) was relatively unresponsive in 1936–37. Moreover, the control lots of the two stocks of Creole included in the 1936–37 trials showed a striking difference in

blooming time. This is most probably attributable to handling before these two stocks reached Beltsville, since all commercial Creoles are grown and harvested under rather closely comparable conditions. The effect of cool storage is cumulative, and Creole $C_2$37 apparently had undergone some cooling before it was received. Additional cool storage applied to a commercial lot already exposed to some similar treatment still shows a significant shortening of the time required for blooming, but the full effect of the treatment is obscured by the failure of the control lot to show the expected delay in flowering. It seems futile, therefore, to attempt to determine varietal differences in response to these treatments by using material so diverse in origin and prior handling. Genetic differences in capacity to respond presumably occur, but they are hopelessly confounded with physiological differences due to different sites of production and methods of handling.

Storage at 50° F. was in general more effective in inducing earliness than either 40° or 32° for comparable intervals, but the differences are not consistently significant. This point is suggested in the data in table 2, but will be shown more clearly later. Storage at 50° or 40° for 10 weeks induced more prompt blooming for all lots than 5 weeks' storage at the same temperature. The lots stored 17 weeks at 32° all bloomed much more promptly than comparable lots stored for shorter periods. These lots (No. 10) of course received the additional stimulus of higher greenhouse temperature, which rises beyond control in May at Beltsville, Md.

The time required for the plant to emerge from the soil shows great irregularity. Some stocks (Creole $C_1$37, Early Erabu, Erabu, and Harrisii) emerged more promptly after storage treatment. This was a material advantage in Harrisii, which was slow to emerge when not placed in cool storage. Apparently cool storage reduces the importance of the common practice of starting bulbs at a low temperature, which is raised after growth has started. Storage at 50° F. for 10 weeks was followed by prompt emergence of all varieties except Harrisii. Both shoot and root growth occurred at this temperature, but the resulting plants were not salable because the etiolated shoots developed in storage never expanded to normal size. Moist storage at 50° should not be practiced except for short intervals, such as 4 to 6 weeks, as a means of inducing extreme earliness.

That cool storage has a tendency to induce flowering while the plants are short is shown in table 2 by significant shortness of plants in all lots stored 10 weeks or more at 32°, 40°, or 50° F. The 5-week storage intervals in general showed no significant reduction in height. There was also a tendency for cool-stored bulbs to produce fewer flowers than the controls. This point is illustrated in table 2 by the number of flowers in lots stored 10 weeks or more, which is usually, but not always, significantly lower than the number for the corresponding control lot. The effects of cool storage on both height and number of flowers are better illustrated in data to be discussed in a later section (p. 328).

The interval between the first and last flowers on a single plant, which was visualized as an index of duration of attractiveness of a lily as a pot plant, was also measured. The data have not proved very helpful, however, so they are not included. They appear to be dependent on the number of flowers per plant as well as on the lasting

qualities of individual flowers and on other factors. Low values obtained for the lots stored for 10 weeks at 50° F. are largely a reflection of a reduced number of flowers in these lots.

The mean size of flower is a very stable characteristic of the variety as compared with other characteristics shown in table 2. Very few differences in this column show evidence of significance.

1937–38 EXPERIMENT

CREOLE VARIETY

Since comparisons of varietal response had not proved enlightening, the 1937–38 experiments were confined chiefly to the Creole variety, which could be obtained shortly after digging, with the addition of a simple interval test with Harrisii designed to extend the bloom of this variety over 2 or 3 months. Both varieties were received on August 26 direct from producing areas with no exposure to cool storage before shipment. Storage trials were set up immediately on receipt of the stocks.

The experiments were intended to provide comparisons between moistened-peat and dry-soil storage and also to afford an opportunity to examine further the effect of the length of the storage interval. The 40° and 50° F. storage trials were not carried beyond 15 weeks' duration, since the 1936–37 results had shown that these temperatures, if long continued, were likely to produce unfavorable effects. The 32° storage was continued up to 60 weeks to determine the tolerance of the Creole variety to this temperature, which in previous trials had appeared least deleterious in its effects on forcing quality. It was hoped that the bloom of the Creole variety could be extended throughout an entire calendar year by suitable storage manipulation. This result was indeed attained, but the average forcing quality of the 7- to 9-inch bulbs was poor after 60 weeks' storage at 32°.

The 1937–38 trials with the Creole variety are summarized in table 3. It will be noted at once that the number of days required to bloom is very greatly reduced by all storage practices included in these trials. In fact, all 5 weeks' treatments, all 10 weeks' treatments, and 2 of the 15 weeks' treatments were significantly earlier in actual date of blooming than the control lot, which flowered during Easter week. These differences are readily visualized by reference to figure 1. These clear-cut differences, which contrast with the less satisfactory results of the previous year, are attributed to the fact that this Creole stock was received before it had been subjected to previous storage.

The earliest bloom was produced by lot 4, which was stored 5 weeks at 50° F. in moist peat. The flowers were of satisfactory size and quality but sharply reduced in number (3.6 per plant), and the plants were significantly shorter than the controls; however, they were very acceptable Easter lilies for February. Lot 7, stored dry at 50° for 5 weeks, bloomed about 14 days later, with no significant improvement in the number of flowers. Longer storage at 50° (10 weeks and 15 weeks, dry) reduced the number of flowers to about two per plant. It seems clear that long storage at 50° is undesirable, and that this temperature is useful only in inducing extreme earliness of bloom.

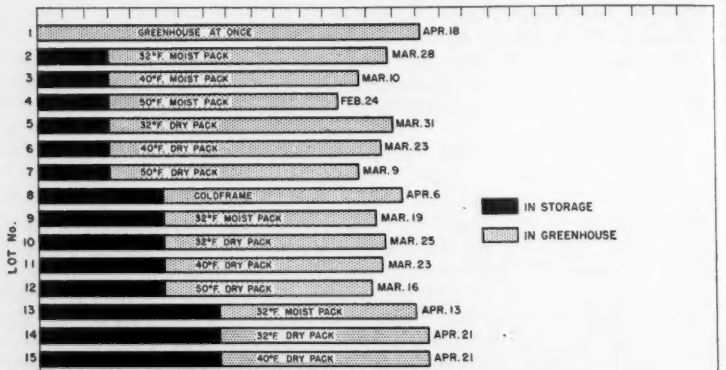
LOT No.
1 GREENHOUSE AT ONCE APR. 18
2 32°F. MOIST PACK MAR. 28
3 40°F. MOIST PACK MAR. 10
4 50°F. MOIST PACK FEB. 24
5 32°F. DRY PACK MAR. 31
6 40°F. DRY PACK MAR. 23
7 50°F. DRY PACK MAR. 9
8 COLDFRAME APR. 6
9 32°F. MOIST PACK MAR. 19
10 32°F. DRY PACK MAR. 25
11 40°F. DRY PACK MAR. 23
12 50°F. DRY PACK MAR. 16
13 32°F. MOIST PACK APR. 13
14 32°F. DRY PACK APR. 21
15 40°F. DRY PACK APR. 21
16 50°F. DRY PACK APR. 10
17 32°F. DRY PACK MAY 8
18 32°F. DRY PACK JUNE 18
19 32°F. DRY PACK SEPT. 20
20 32°F DRY PACK JAN. 27, 1939
IN STORAGE
IN GREENHOUSE
AUG. 16 SEPT. 1 SEPT. 15 OCT. 1 OCT. 15 NOV. 1 NOV. 15 DEC. 1 DEC. 15 JAN. 1 JAN. 15 FEB. 1 FEB. 15 MAR. 1 MAR. 15 APR. 1 APR. 15 MAY 1 MAY 15 JUNE 1 JUNE 15 JULY 1 JULY 15 AUG. 1 AUG. 15 SEPT. 1 SEPT. 15 SEPT. 30
1937
1938

FIGURE 1.—Chart of 20 storage trials with Creole Easter lilies in 1937–38, showing relative length of time in storage and in the greenhouse and mean flowering data.

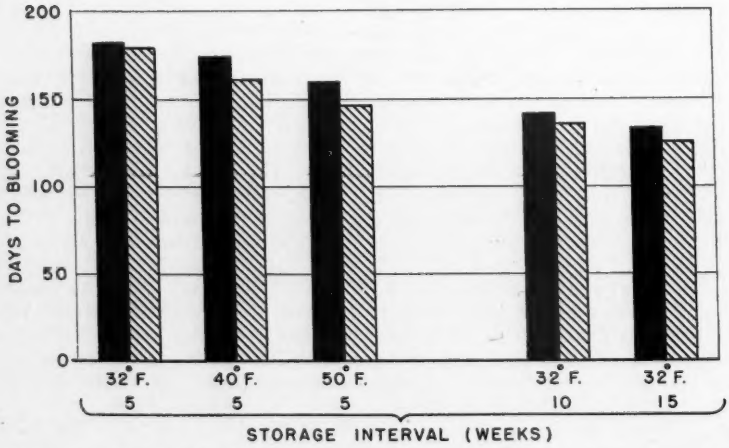

FIGURE 2.—Charts showing comparative effects of storage in dry pack and in moist pack (shaded) on days to blooming in Creole Easter lilies in the 1937–38 trials.

TABLE 3.—*Effect of storage treatment on time of blooming and quality of flowers of the varieties Creole and Harrisii in 1937–38*

CREOLE $C_1$38 (MEAN CIRCUMFERENCE OF BULBS, 7.64±0.05 INCHES)

Lot No.[1]	Storage treatment: Temperature	Storage treatment: Interval	Storage treatment: Pack	Date planted in greenhouse	Average date of blooming	Days to blooming [2 3]	Days to emergence [2 3]	Height [3]	Flowers per plant [3]	Leaves per plant [3]	Size of flower [3] (length by diameter)
	°F.	Weeks		1937	1938	Number	Number	Inches	Number	Number	Index number
1 [4]				Aug. 26	Apr. 18	235.3±0.65	42.0±0.57	24.6±0.83	8.5±0.19	162.7±3.19	25.5±0.54
2	32	5	Moist	Sept. 30	Mar. 28	179.1± .93	31.9± .87	21.1± .55	5.1± .16	102.5±1.51	25.5± .48
3	40	5	do	do	Mar. 10	160.9± .78	25.6± .55	20.5± .55	4.7± .11	84.9±1.21	25.2± .68
4	50	5	do	do	Feb. 24	146.8±1.00	16.0± .55	19.1± .46	3.6± .17	69.5±1.42	25.6± .59
5	32	5	Dry	do	Mar. 31	182.0± .91	33.9± .75	22.6± .74	5.2± .10	101.5±1.18	25.0± .59
6	40	5	do	do	Mar. 23	174.2± .72	35.5± .95	20.0± .57	4.2± .19	78.9±1.63	25.8± .49
7	50	5	do	do	Mar. 9	160.4±1.07	26.6± .70	19.2± .44	3.6± .19	73.8±1.40	25.1± .52
8	[5]	10		Nov. 4	Apr. 6	153.2± .96	[6]	19.3± .60	6.0± .18	99.6±2.58	25.2± .48
9	32	10	Moist	do	Mar. 19	135.2±1.06	25.4± .49	24.1± .56	4.8± .14	70.9±1.51	26.5± .48
10	32	10	Dry	do	Mar. 25	141.4± .65	30.1± .60	26.0± .65	4.5± .17	63.6± .89	26.4± .46
11	40	10	do	do	Mar. 23	139.1± .95	31.5± .98	24.8± .44	4.2± .19	52.4± .70	26.9± .45
12	50	10	do	do	Mar. 16	131.7±1.05	29.4±1.50	18.9± .57	2.2± .22	46.9± .72	27.6± .67
13	32	15	Moist	Dec. 9	Apr. 13	125.5± .40	26.2± .61	28.2± .52	5.2± .11	49.0±1.05	27.5± .25
14	32	15	Dry	do	Apr. 21	132.8± .74	32.2± .85	24.4± .48	4.8± .12	50.3± .77	27.2± .18
15	40	15	do	do	do	133.3± .78	38.5±1.04	20.2± .56	3.0± .14	41.1± .79	26.8± .37
16	50	15	do	do	Apr. 10	121.8±1.09	26.9±1.27	15.8± .50	1.8± .13	44.3± .66	27.0± .48
				1938							
17	32	20	do	Jan. 13	May 8	115.1± .47	34.4± .81	20.0± .50	4.3± .13	41.3± .72	27.8± .17
18	32	30	do	Mar. 24	June 18	86.5± .73	26.5± .68	18.4± .46	3.9± .19	32.8±1.37	25.1± .30
19	32	45	do	July 7	Sept. 20	74.6±1.42	15.8± .62	20.2± .32	2.6± .13	40.5± .95	23.6± .14
					1939						
20	32	60	do	Oct. 20	Jan. 27	99.2±5.80	21.1± .99	17.0± .61	2.0± .17	24.2± .72	24.4± .96

HARRISII H38 (MEAN CIRCUMFERENCE OF BULBS, 7.98±0.23 INCHES)

				1937	1938						
1 [4]				Aug. 26	Mar. 15	201.0±1.39	48.1±0.57	17.8±0.43	6.2±0.24		30.3±0.44
2	40	5	Dry	Sept. 30	Apr. 4	186.2±1.01	43.3± .79	21.2± .48	5.4± .13		30.8± .33
3	40	10	do	Nov. 4	Apr. 11	158.3± .76	36.6± .76	25.0± .34	4.7± .20		32.8± .38
4	40	15	do	Dec. 9	May 7	149.7± .75	43.8± .87	22.1± .35	3.7± .19		34.3± .27

[1] 25 plants in each lot of Creole and 40 plants in each lot of Harrisii.
[2] From date of planting in greenhouse.
[3] Mean and standard error.
[4] Bulbs planted in greenhouse on arrival.
[5] Unheated coldframe.
[6] All bulbs up when brought into greenhouse.

A comparison of five pairs of lots stored in moistened peat and dry-soil pack at the same temperatures and intervals (fig. 2) shows that the moist pack induced earlier bloom. From these five pairs the mean difference in time of blooming is 8.66 days, and the odds exceed the 5-percent level and approach the 1-percent level of significance. The individual differences in time of blooming are each statistically significant, but are too small to assume any practical significance except where maximum earliness is desired. These differences in general could have been made up more conveniently by raising the forcing temperature, which is considerably less trouble (although possibly more expensive) than repacking in moist peat. Where maximum earliness is required the two methods could be combined. All the writer's trials thus far have been run at a comparatively cool forcing temperature. An increase of 10° F. during January presumably would have advanced the earliest flowering date (February 24) still further.

Further consideration of the mean dates of bloom in table 3 reveals that lots stored at 32° F. in dry pack, i. e., in original containers, were the last, or among the last, to bloom in each storage-interval group. The differences between storage at 32° dry and that at 50° dry are always significant, but storage at 32° does not always differ significantly in effect from that at 40°. Similar comparison of the data for the number of flowers from these lots shows that 32° storage was always followed by more flowers per plant than 50° storage. The differences between 32° and 40° storage are smaller and not all are significant, but when real differences appear they show more flowers from lots stored at 32°. When t tests are applied to four available pairs of measurements, lots stored at 32° and 40° are found to flower significantly later than those at 50°, and lots at 32° exceed those at 50° in number of flowers by a highly significant difference. These data show 32° to be the best of the temperatures tested for holding Easter lilies for long periods with minimum deterioration of forcing quality.

The effect of progressively longer intervals of storage at 32° F. in dry-soil pack is shown in the bar charts in figure 3, plotted from data included in table 3. It appears from the chart that the effect of continued storage in stimulating early bloom is cumulative. The form of the chart suggests a curvilinear relation, in which additional increments of storage have a reduced effect in stimulating earliness. The fact that the suggested curve is obviously not a smooth one is perhaps explainable as due to temperature differences during forcing. The decline in number of flowers shows a similar trend but is less smoothly expressed. The effect of storage at 32° on height is not uniform in trend, but reference to table 3 will show a rather consistent decline in height with progressively increasing periods of 50° dry storage.

The data for days to emergence are also presented in figure 3, *B*, and table 3. All stored lots emerged more promptly than the control lot, and the differences are significant though not large. The mean difference in time of emergence between moist and dry storage at the same temperature and storage duration is 6.63 days, a significant value. The five individual differences are always in favor of moist storage but are not always individually significant. Longer periods of

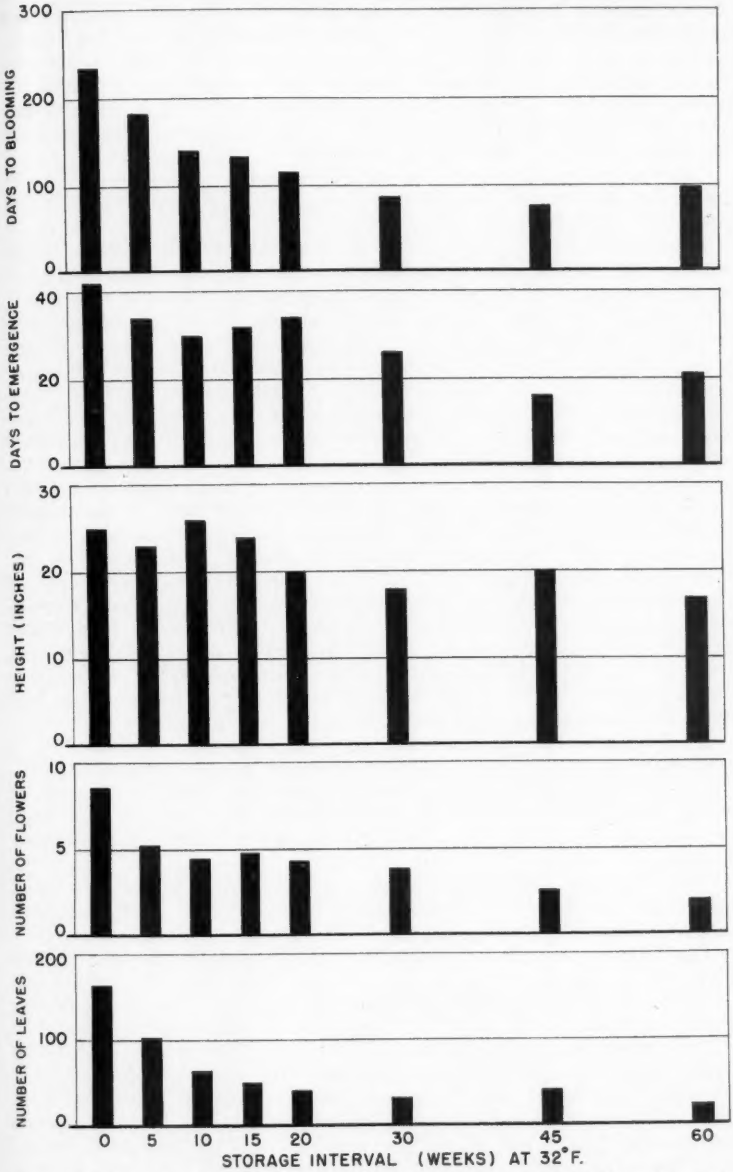

FIGURE 3.—Charts showing effect of duration of storage of the bulbs in dry sandy loam at 32° F. on days to blooming (*A*), days to emergence (*B*), height (*C*), number of flowers (*D*), number of leaves (*E*), and relative size of bloom (*F*). in the Creole Easter lily in the 1937–38 trials.

dry storage at 40° F. show slower emergence than comparable lots stored similarly at 32° or 50°, but these differences are significant only in the 15-week interval. The *t* test shows no significant differences in days to emerge between 32° and 40°, 40° and 50°, nor 32° and 50° storage when the four available comparisons are grouped. In general, the Creole variety is not difficult to get up, but cool storage and the presence of moisture hasten its emergence.

Decline in height of the plants after storage treatment is notable chiefly in the longer storage intervals at 50° F., but it is also evident after very long storage at 32°.

The number of flowers per plant drops sharply from the control value in all stored lots, the nearest approach to the control appearing in the coldframe lot. This drop in number is more rapid at 40° F. than at 32°, and more rapid at 50° than at 40°, in all comparisons available. A highly significant *t* value is found for the four comparisons between 32° and 50°, but other comparisons, 32° as compared with 40°, and 40° as compared with 50° yield nonsignificant values. Even in 32° storage the decline in number of flowers is the chief limiting factor determining the length of time that bulbs of the 7- to 9-inch size may be held (fig. 3, *D*) without too serious effects in forcing quality.

The number of leaves developed per plant shows a close parallel in decline to the number of days required to bloom and to the number of flowers developed per plant (fig. 3, *A*, *D*, *E*). Again the decline is greater at 50° F. storage than at 32°, greater at 40° than at 32°, and greater with progressive intervals at the same storage. Not all the individual differences are significant, but general trends are evident. Applying *t* tests to four available comparisons, lots stored at 32° show a significantly higher leaf count than lots stored at either 40° or 50°. In addition to the decline in number of leaves, a decline in size of the lower leaves becomes more and more evident with increasing storage intervals.

No measurements of leaf size have been made, but the effect is evident in figures 4 and 5. Bulbs stored 15 weeks or more have incompletely developed lower leaves. Some of those stored for 60 weeks developed leaves irregularly; the lowermost leaves were reduced to scales, and some midway up the stem also remained rudimentary in form.

Size of flower again appears as a relatively stable characteristic, with very few differences approaching the 5-percent level of significance, and no lot differing significantly from the control lot.

The Creole lot which was stored dry for 60 weeks at 32° F. was scattered in the greenhouse, five plants at each of five situations (blocks), to afford a measure of place effects in this particular house. Two lots were placed near the inner end of the house, nearest the steam pipes most frequently used, two lots at the opposite end of the house nearest the outside door, and the fifth lot in the center. On analysis of variance, no significant differences were found between blocks for any of the six characteristics measured. The two blocks nearest the inside door bloomed earlier, but not significantly earlier, than the remaining three blocks. Although there appeared to be a temperature gradient from the inner to the outer end of the greenhouse, the writer failed to demonstrate a significant difference due to position in this trial. It is thought that the place effects measured

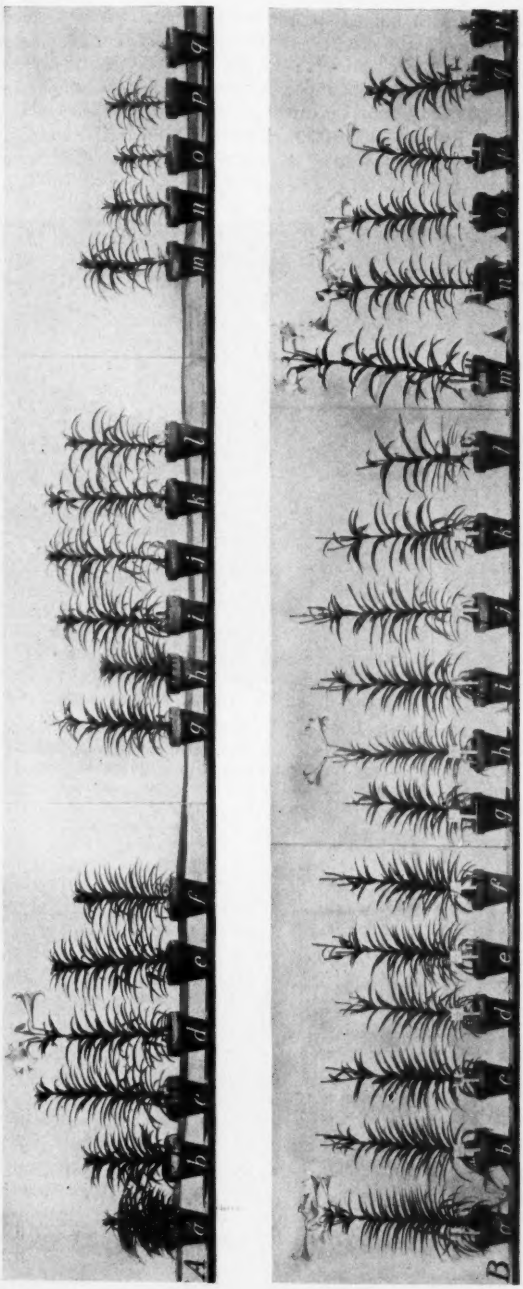

FIGURE 4.—Representative plants from storage experiments with Creole Easter lilies in 1937–38, photographed February 12 (*A*) and April 23 (*B*), 1938. Storage treatments in each row: (*a*) None; (*b*) 5 weeks in moist pack at 32° F.; (*c*) 5 weeks in moist pack at 40°; (*d*) 5 weeks in moist pack at 50°; (*e*) 5 weeks in dry pack at 32°; (*f*) 5 weeks in dry pack at 40°; (*g*) 5 weeks in dry pack at 50°; (*h*) 10 weeks in coldframe; (*i*) 10 weeks in moist pack at 32°; (*j*) 10 weeks in dry pack at 32°; (*k*) 10 weeks in dry pack at 40°; (*l*) 10 weeks in dry pack at 50°; (*m*) 15 weeks in moist pack at 32°; (*n*) 15 weeks in dry pack at 32°; (*o*) 15 weeks in dry pack at 40°; (*p*) 15 weeks in dry pack at 50°; (*q*) 20 weeks in dry pack at 32°; (*r*) 30 weeks in dry pack at 32° (planted March 24). Note the trend toward fewer leaves and fewer flowers from the longer storage intervals.

here represent a maximum, since the plants were grown October 20 to February 22, when steam was used regularly and loss of heat through the outside door was greatest, and the lots were so distributed as to include maximum temperature differences in the house. Although the treatments were not randomized through the greenhouse, place effects probably do not seriously affect the general conclusions presented in this paper, but they may account for some of the irregularity in trends shown in figure 3.

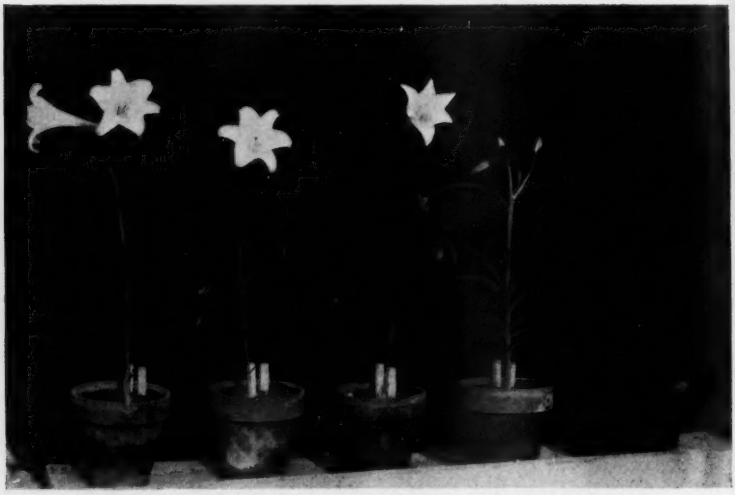

FIGURE 5.—Creole Easter lilies in bloom January 12, 1939, after 60 weeks' storage in dry soil at 32° F. and 94 days in the greenhouse. Note reduced number of leaves and flowers and imperfect leaf development. For comparison, one plant (at extreme right) is from a new crop commercial Creole bulb not subjected to cool storage, after 97 days in the greenhouse.

HARRISII VARIETY

The treatments and data for the 1937–38 Harrisii stock are shown in table 3. These data are of interest chiefly because of the differences shown between the Harrisii and Creole varieties when treated alike, and because of certain marked departures in behavior of the Harrisii stock under discussion from that of the Harrisii stock of 1936–37. If the data for Harrisii are compared with those for Creole (table 3), it is found that in untreated lots (lot 1) Harrisii was a month earlier, but after 5 weeks in dry storage at 40° F. Creole was the earlier; after 10 and 15 weeks Creole was again the earlier. Thus Harrisii proved earlier but less responsive to cool storage than Creole in these samples. None of the storage treatments applied to Harrisii (40° dry, for 5, 10, 15 weeks) advanced the actual date of flowering, while Creole exposed to the same treatment flowered in advance of the control after both 5 and 10 weeks' storage, and after 15 weeks it flowered at essentially the same time as the control lot. However, all three intervals at 40° shortened the number of days required to bloom after

planting in both varieties. In Harrisii the number of flowers per plant declined steadily with progressively longer storage intervals, with all differences significant. After comparable storage intervals the number of Creole flowers dropped sharply after 5 weeks' storage, remained unchanged after 10 weeks, then dropped lower after 15 weeks. In Harrisii the size of flower increased steadily with increasing time in storage; in Creole no such change was evident. Incidentally, the significant increase in Harrisii is believed to be due not to storage, but to environmental factors that became successively more favorable for growth and development as these lots flowered. Such comparisons between these two varieties are probably sound, since the two presumably matured and were harvested at the same time and were without storage treatment before arrival. It is still conceivable that the environmental effects of the two production areas were responsible for the differences observed, but this evidence is the best the writer has obtained thus far to show that commercial stocks differ in capacity to respond to cool storage.

A comparison of the Harrisii stock of 1936–37 (table 2) with that of 1937–38 (table 3) shows some striking differences. The 1936–37 stock was purchased through a New York dealer and reached Beltsville one month later in the season than the 1937–38 stock, which was bought direct from a producer in Bermuda. The bulbs in the two lots were of the same size and grade, and received similar treatment by the writer except that more varied treatments were imposed on the earlier lot. The 1937–38 untreated lot emerged much more promptly, bloomed earlier, and produced twice as many flowers per plant as the corresponding lot of the preceding year. The response to storage was similar in the 2 years; in neither year did any stored lot bloom before the control lot. It seems probable that the 1936–37 stock of Harrisii received some unfavorable treatment before reaching Beltsville. Since the evidence presented above indicates that Harrisii is somewhat divergent from other Easter lilies in response to storage, it is possible that the variety suffers adverse effects from routine bulb-storage practices.

CORRELATION AMONG THE CHARACTERISTICS MEASURED

Cool storage is utilized chiefly to advance or retard the date of flowering in Easter lilies, but a number of other characteristics recorded in this study such as number of leaves and number of flowers, appeared to be strongly modified by the treatments. It is of considerable interest to learn how closely these characteristics are correlated, or how strong is their tendency to respond together to storage treatments. Simple correlation coefficients have been computed for all possible pairs of the seven characteristics measured in the Creole variety and of the six measured in the Harrisii variety in the 1937–38 trials.

Because of the large number of degrees of freedom, even very low correlation coefficients have statistical significance; however, comparatively few pairs of characteristics show close association. The square of the value of r is sometimes used as an index of percentage correlation. For example, in the Creole variety, 52 percent of the variation in height, 37 percent of that in number of flowers, 77 percent of that in number of leaves, and 30 percent of that in duration of bloom are found associated with days to blooming. Consequently

treatments designed to accelerate bloom may be expected to reduce the number of leaves, and they are also likely to reduce height, number of flowers, and duration of bloom. Number of flowers shows a 57-percent correlation with number of leaves. Few of the other relationships are sufficiently close to assume practical importance. Size of the flower shows little or no correlation with other factors studied in the Creole variety.

The Harrisii variety included only 160 recorded individuals, and less varied treatments were imposed on these than on Creole. Few of the correlation values are high, although many are significant. Some differences in varietal tendency appear. Accelerated flowering is associated to the degree of 25 percent with taller plants in Harrisii, but to 52 percent with shorter plants in Creole. There is a 25-percent tendency for late-blooming plants of Harrisii to bear larger flowers, but no relation at all appears between days to bloom and size of flower in Creole. As explained above, there is some reason to believe that Harrisii displays different responses to storage from those of Creole, but the differences found here may result chiefly from sampling during part of the calendar year with Harrisii (March to May) and during the entire year (February to January) with Creole.

1938–39 EXPERIMENT

No formal storage trials were planned for the season of 1938–39 but the performance of one lot stored and forced for early vegetative growth for other purposes seems of sufficient interest to report. Seedling Easter lilies were dug at Charleston, S. C., on July 13, 22 months from seed. At this time some of the tops were well matured and some still green. A random selection of five seedlings from each of five progenies made up the experimental lot. The bulbs were shipped to Beltsville, Md., and stored in moistened peat at 50° F. from July 22 to September 2, or 6 weeks. On the latter date they were potted into 4- or 6-inch pots according to size, and were forced under the conditions of previous trials. A large number produced flowers at far earlier dates than any lot of new crop Easter lilies previously forced. The range of flowering dates was December 10 to January 17 for the 25 bulbs from which data were collected. The mean flowering date was January 2. The plants required 121.4 ± 2.25 days to bloom, and bore 2.6 ± 0.32 flowers per bulb, at a height of 18.3 ± 1.24 inches above the soil.

These data suggest that Easter lilies for December and January bloom should be produced from new crop bulbs rather than from stored bulbs of the previous season, provided the new crop bulbs can be prepared safely and consistently. The new crop lilies compared very favorably with stored Creoles of the previous season. At least four factors may be utilized in inducing maximum earliness of bloom in new crop Easter lilies: Genetic earliness, early digging, cool storing, and forcing at comparatively high temperatures. Considerably more detailed information on the hazards involved and on the most efficient combination of these factors is needed before new crop lilies will appear on the market at Christmas, but this objective should be attainable.

DISCUSSION

The control of flowering time in bulbous plants by suitable manipulation of storage temperatures has long been in commercial use. Workers at the Wageningen and Lisse laboratories in the Netherlands have made detailed studies of the seasonal changes in bulbs of hyacinth, tulip, and narcissus.[2] The Netherlands workers have determined the temperature requirements for organ formation and for elongation in these bulbs, and have then devised temperature sequences for early and for late forcing.

Pfeiffer [3] studied the successive stages of development of the floral axis in bulbs of Bermuda Harrisii lilies stored at 10° to 13° C. (50° to 55.4° F.) and in Japanese Giganteum stored at 3° C. (37.4° F.). Harrisii bulbs received in August were in the vegetative stage, which persisted to mid-October when the predifferentiation stage of the terminal bud was recognizable. In the variety Giganteum, bulbs received in December already showed evidence of the predifferentiation stage, but floral organs were not recognizable in storage until April 16. Development in storage was slow in both varieties, but the cumulative effect of this development brought more rapid completion of the flower primordia after planting and hence more prompt blooming.

No morphological study of the Creole variety, which was used chiefly in the present study, is available. It may be assumed tentatively, however, that the cool storage treatments were applied mainly prior to differentiation of the floral organs. The early stages of bud development and possibly also the later stage of organ formation may have an optimum temperature near 50° F. The observed advantage of 50° over 32° storage, and some of the differences in performance of the several varieties and of different stocks of the same variety may thus be explainable on the basis of the temperature requirements of successive developmental phases within the bulbs.

Previous studies of the effect of cool storage on the performance of Easter lilies replanted in the field have been made in Bermuda [4] and in Florida. [5] In the Bermuda experiment, bulbs of the Harrisii variety were subjected to 37° F. storage in sand for monthly periods ranging from 0 to 4 months, beginning September 18, about 8 or 10 weeks after harvest. The bulbs subjected to cool storage emerged and bloomed more promptly but produced fewer flowers than controls planted at the start of the storage trial; there was an actual advance over the controls in the flowering date of the lots stored 1 and 2 months. The authors of the Bermuda report stated that the decline in number of flowers per bulb resulted from ordinary storage as well as from cool storage. Shippy [5] found that the Florida strain of Easter lily (similar to Creole and perhaps identical with it) emerges and blooms earlier in Florida field culture after a period of cool storage. A temperature of 40° F. and a packing of dry sand were used. Shippy varied the

[2] PURVIS, O. M. RECENT DUTCH RESEARCH ON THE GROWTH AND FLOWERING OF BULBS. I. THE TEMPERATURE REQUIREMENTS OF HYACINTHS. Sci. Hort. [Wye, Kent] 5: 127–135, illus. 1937.
——— RECENT DUTCH WORK ON THE GROWTH AND FLOWERING OF BULBS. II. THE TEMPERATURE REQUIREMENTS OF TULIPS AND DAFFODILS. Sci. Hort. [Wye, Kent] 6: 160–177, illus. 1938.

[3] PFEIFFER, NORMA E. DEVELOPMENT OF THE FLORAL AXIS AND NEW BUD IN IMPORTED EASTER LILIES. Boyce Thompson Inst. Contrib. 7:311–321, illus. 1935.

[4] BERMUDA DEPARTMENT OF AGRICULTURE. AN EXPERIMENT ON COLD STORAGE OF LILY BULBS. Bermuda Dept. Agr., Agr. Bul. 14: 52–54. 1935.

[5] SHIPPY, WILLIAM B. FACTORS AFFECTING EASTER LILY FLOWER PRODUCTION IN FLORIDA. Fla. Agr. Expt. Sta. Bul. 312, 19 pp., illus. 1937.

digging date and the date and duration of cooling, and included warm storage treatments and bulbs replanted immediately as controls. Bulbs were accelerated in emergence and flowering by cool storage for an interval of 30 days or more but not for a 21-day interval. A few flowers were produced in December, and more in January, from cool-stored bulbs replanted in the field. The most favorable effects in the production of early bloom followed cool storage of 30 days or more applied after the middle of August. Shippy found that fewer flowers were produced from cool-stored lots, but that the increase in number of bulbs was not similarly affected.

The present investigation indicates that 50° F. is more effective in inducing early bloom than lower temperatures, and that moistened peat is slightly superior to dry-sand pack for this purpose. It also appears that all possible factors subject to control and tending to early bloom have not yet been combined. A possible danger in digging before bulbs are sufficiently mature is suggested in one of the writer's tests not here detailed.

However, early bloom is only one goal of Easter lily forcing. It is necessary to provide Easter lilies in every month of the year in response to market demand. The present study shows that this can be done with a single variety and a single digging date, but suggests that better ways should be found for producing fall bloom. After Easter lily bulbs are held for long periods in the least deleterious storage condition known (32° F. dry), they are inferior in forcing performance. Commerical Creole bulbs of the 7- to 9-inch size can be brought to flower from late February to September by simple manipulation of the conditions of storage, and the plants and flowers will be of satisfactory grade. Bulbs of larger size, commonly used for long storing in the trade, will probably produce satisfactory bloom from October to December. Further research on the effects of digging date and forcing temperature should lead to safe provision for November to January bloom from new crop bulbs. The period from September to November probably must be covered by bulbs stored nearly a full year, the practice now followed with Japanese Giganteum. Bulbs of the larger sizes should be used, as is customary when long storage is planned. Present commercial practice now utilizes Erabu for early bloom and Giganteum for late bloom. Although much of the year can be covered with a single variety such as Creole, it is perhaps doubtful whether a single variety can be made to perform most satisfactorily over the whole period.

SUMMARY

Storing Easter lily bulbs for 5 or more weeks at 32° to 50° F. accelerates flowering. Storage at 50° is more effective than at 40° or 32° in producing this effect. Bulbs packed in moistened peat moss during the storage interval flowered slightly in advance of those packed in dry soil or sand.

Storage for 10 weeks or more at 50° F. is distinctly deleterious, reducing the number of flowers and the number of leaves per plant. Similar effects result from storage for longer intervals at 40°. Storage at 32° in dry sand kept Creole Easter lilies with less deleterious effects on forcing quality than at 40° or 50°. After 45 weeks at 32° the

quality was barely acceptable, and after 60 weeks the average performance of 7- to 9-inch bulbs was poor.

Effects of cool storage on time required for the plants to emerge from the soil and on the height of the plant are less consistently evident, but when differences appear they are in the direction of decline from the level shown by control plants not subjected to storage. The size of the flower is a relatively constant characteristic of the variety, and is little affected by storage of the bulbs.

It appears probable that new crop bulbs of early varieties can be forced for Christmas by suitable combination of early harvest and cool storage treatment.

Cool storage of Easter lily bulbs is a convenient primary control over time of flowering. The customary control factor, forcing temperature, can be used as a fine adjustment after this primary control has been applied.

A STUDY OF VARIOUS METHODS OF PRESERVING LEGUMES AND OTHER FORAGES BY ENSILING[1]

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INTRODUCTION

During the past 20 years there have appeared three principal methods of preserving legumes by ensiling. These are the A. I. V. process developed by A. I. Virtanen (*3*, *37*, *38*),[2] the molasses method first used at the Kansas Agricultural Experiment Station (*27*, *33*, *34*), and the phosphoric acid method that seems to have been first proposed by Virtanen (*37*). The present writers have been interested in the preparation of silages from a large variety of forage crops by the above methods and by a number of other less well-established procedures.

In order to decide whether a particular procedure was suitable for trial on a silo scale it has been the practice at this station to try out such a procedure first on bottle or barrel lots. The results of the preliminary trials proved to be very reliable guides as to what might be expected on a larger scale. For example, alfalfa put up by the A. I. V. method has invariably been good in bottles, barrels, or silos. The same has been true of alfalfa silage made with phosphoric acid or molasses when these were used at the rate of 30 and 60 pounds per ton, respectively. Poor silage has been the rule, irrespective of the type of container, when no preservative was added. With bottle or barrel lots particular care must be observed to pack the forage tightly so as to exclude air.

The silages were analyzed for dry matter, pH value, carotene, and in some cases ammonia and total nitrogen as an additional check on preservation. The analyses of most interest are pH value, which is a good criterion of preservation, and β-carotene, which is necessary for the production of winter milk of high vitamin A potency.

In cases where sufficient material was available, feeding tests were made with cows of the University herd to determine the palatability of the silage.

PREPARATION OF SILAGES

BOTTLE SILAGES

The bottle silages were prepared by mixing by hand 1 kg. of the freshly cut and chopped forage with the appropriate amount of preservative. A quart milk bottle was filled with the material and closed with a rubber stopper containing a Bunsen valve. The material must be pressed in very tightly with a round flat-end stick, such as a hammer handle, to prevent mold growth. With material

[1] Received for publication August 9, 1940. Presented in part before the Division of Agricultural and Food Chemistry of the American Chemical Society at the September 1940 meeting at Detroit, Mich.

[2] Italic numbers in parentheses refer to Literature Cited, p. 346.

containing 25 percent or less of dry matter, an index of good packing is the accumulation of juice on top of the material. A bottle holds approximately 750 gm. of silage. Table 1 gives the analytical results on the bottle silages.

TABLE 1.—*The effect of various preservatives on the composition and quality of silage in bottle experiments*

Lot No.[1]	Material [2]	Treatment	Preservative added per ton	Dry-matter content	pH value	Carotene per gram of dry matter	Quality of silage [3]
				Percent		γ	
5-9	Alfalfa (5)	None		25.3	5.1	168	Poor.
5-0	Alfalfa	A. I. V	70 liters of 2N acid.	27.2	4.0	.....	Good.
8-5	do	Corn meal	30 pounds	22.2	5.5	244	Poor.
8-7	Alfalfa (2)	do	60 pounds	22.0	5.3	182	Do.
8-9	Alfalfa	do	90 pounds	24.2	5.3	231	Do.
8-11	Alfalfa (2)	do	120 pounds	25.2	4.6	174	Fairly good.
9-37	Alfalfa	Soured-whey concentrate.	70 pounds	35.5	4.5	190	Do.
9-35	do	do	140 pounds	32.0	4.5	207	Good.
9-33	do	do	280 pounds	32.5	4.3	200	Do.
9-28	do	Sulfamic acid	50 pounds	28.0	4.6	181	Fair.
8-65	do	do	80 pounds	20.5	4.5	298	Do.
8-64	do	do	160 pounds	21.0	3.4	327	Do.
8-1	do	Whey powder	20 pounds	24.6	4.7	225	Poor.
8-2	do	do	80 pounds	24.3	4.3	207	Good.
6-0	Alfalfa and corn (1-1)	None		23.0	4.4	Trace	Fairly good.
6-3	Alfalfa and rye (1-1)	do		21.0	4.6	.....	Fair.
6-2	Corn	do		20.0	3.7	.....	Good.
9-13	Clover and timothy (2).	do		24.7	5.6	241	Poor.
9-26	do	A. I. V	70 liters of 2N acid.	25.0	3.9	156	Good.
9-16	do	Phosphoric acid	30 pounds	26.5	4.0	212	Do.
9-20	Clover and timothy	Molasses	70 pounds	25.0	4.3	208	Do.
9-22	do	Soured molasses	60 pounds	26.0	4.0	210	Do.
8-14	Clover (red)	A. I. V	70 liters of 2N acid.	25.0	3.9	68	Do.
8-15	do	Phosphoric acid	30 pounds	26.1	4.0	102	Do.
8-16	do	Molasses	70 pounds	26.5	4.2	80	Do.
8-17	Grass (lawn)	A. I. V	70 liters of 2N acid.	24.5	3.9	196	Do.
8-18	do	Phosphoric acid	30 pounds	25.0	4.1	162	Do.
8-19	do	Molasses	70 pounds	26.2	4.3	152	Do.
9-1	Oats and peas	None		18.2	4.5	340	Fair.
9-11	do	A. I. V	70 liters of 2N acid.	16.5	3.8	371	Good.
9-4	do	Phosphoric acid	30 pounds	17.0	3.9	363	Do.
9-8	do	Molasses	70 pounds	18.0	4.0	371	Do.
6-18	Sweetclover	None		35.5	4.5	.....	Poor.
6-19	do	Molasses	40 pounds	35.5	4.3	.....	Good.
6-20	do	do	100 pounds	35.5	4.2	.....	Do.
7, 8-0	Sudan grass (2)	None		20.2	5.0	.....	Poor.
7-1	Sudan grass	A. I. V	50 liters of 2N acid.	24.0	4.4	.....	Good.
7-2	do	Molasses	70 pounds	26.8	4.1	.....	Do.

[1] To aid in comparing various lots, the first number refers to the year and the second to the sample, e. g., 5-9 refers to 1935 bottle No. 9.
[2] Figures in parentheses denote the number of samples averaged.
[3] Quality based on appearance, odor, and chemical analyses.

BARREL SILAGES

The barrel silages were prepared as follows: 200 pounds of the freshly cut and chopped forage were mixed with the correct amount of preservative and the mixture was put into a wooden barrel. The material was well tramped down during the filling. The upper hoops were left off the barrel during the packing, and after the barrel was tightly packed to above the top the head was forced in and the hoops were replaced. Table 2 gives the analytical data on the barrel silages.

TABLE 2.—*The effect of various preservatives on the composition and quality of silage in barrel experiments*

No.[1]	Material [2]	Treatment	Preservative added per ton	Dry-matter content	NH_3–N as proportion of total N	pH value	Carotene per gram of dry matter	Quality of silage
				Percent	*Percent*		γ	
5–0	Alfalfa	None		21.6	45.0	5.0		Poor.
5–10	do	do		22.6		4.5	65	Do.
6–0	do	do		31.1	18.1	4.6	158	Fair.
7–1	do	do		23.5	8.8	4.6	164	Do.
8–1	do	do		23.2	7.4	4.3	162	Good.
8–10	do	do		26.2	14.5	4.7	130	Poor.
9–1	do	do		25.2		5.3	77	Do.
9–2	do	do		26.0		5.3	18	Do.
7–14	do	Wilted, 27 percent dry matter		27.3	13.4	4.9	133	Do.
8–3	do	Wilted, 28 percent dry matter		27.8	11.6	4.9	132	Do.
7–17	do	Wilted, 33 percent dry matter		33.3	9.4	4.9	109	Do.
8–5	do	Wilted, 38 percent dry matter		37.7	14.5	4.8	125	Do.
9–6	do	Wilted, 42 percent dry matter		42.0		4.9	176	Do.
6–1	do	Wilted, 46 percent dry matter		46.4	9.1	4.5	126	Fair.
9–8	do	Wilted, 48 percent dry matter		44.0		4.6	103	Do.
6, 7–0	Alfalfa (5)	A. I. V.		26.4	5.0	3.7	200	Good.
5–1	Alfalfa	Molasses	10 pounds	21.6	35.2	5.2		Poor.
5–2	do	do	20 pounds	21.6	21.9	5.2		Do.
5–7	Alfalfa (5)	do	40 pounds	27.5	8.1	4.1	138	Good.
6–9	Alfalfa (9)	do	60 pounds	26.9	8.3	4.1	156	Do.
6–7	Alfalfa (4)	do	80 pounds	30.1	6.9	3.9	157	Do.
5–6	Alfalfa (2)	do	100 pounds	27.5	6.9	3.8	148	Do.
5–3	Alfalfa	do	200 pounds	21.6	4.1	3.7	146	Do.
7–16	do	Molasses, wilted, 27 percent dry matter.	80 pounds	28.4	8.2	3.9	173	Do.
8–4	do	Molasses, wilted, 28 percent dry matter.	60 pounds	29.9	11.6	4.1	164	Do.
7–19	do	Molasses, wilted, 32 percent dry matter.	80 pounds	32.0	6.3	4.0	175	Do.
7–18	do	do	80 pounds	32.4	5.0	4.1	120	Do.
8–7	do	Molasses, wilted, 38 percent dry matter.	60 pounds	34.5	8.0	4.2	135	Do.
7–15	do	do	80 pounds	36.4	4.2	3.9	143	Do.
8–6	do	do	60 pounds	38.7	8.3	4.1	142	Do.
9–7	do	Molasses, wilted, 42 percent dry matter.	40 pounds	42.0		4.6	174	Poor.

[1] To aid in comparing various lots, the first number refers to the year and the second to the sample, e. g., 5–0 refers to 1935 barrel No. 0.
[2] Figures in parentheses denote the number of samples analyzed.
[3] Quality based on appearance, odor, and chemical analyses.

TABLE 2.—*The effect of various preservatives on the composition and quality of silage in barrel experiments*—Continued

No.	Material	Treatment	Preservative added per ton	Dry-matter content	NH_3-N as proportion of total N	pH value	Carotene per gram of dry matter	Quality of silage
				Percent	*Percent*		γ	
9-9	Alfalfa	Molasses, wilted, 46 percent dry matter.	30 pounds	45.8		4.5	138	Fair.
6-2	do	do	40 pounds	45.5	6.7	4.2	70	Good.
6-6	do	do	80 pounds	47.0	6.5	4.1	103	Do.
7-12	do	Phosphoric acid	100 liters of 2N acid	25.6	4.6	3.7	114	Do.
7-13	do	do	150 liters of 2N acid	26.4	2.7	3.5	124	Do.
7, 9-4	Alfalfa (2)	Whey powder	40 pounds	26.8	6.1	4.2	172	Do.
7, 9-5	do	do	80 pounds	28.1	5.4	4.0	176	Do.
9-10	Alfalfa	Corn meal	40 pounds	26.5	5.0	5.0	54	Poor.
9-12	do	do	80 pounds	26.8		4.9	42	Do.
9-14	do	do	120 pounds	27.7		4.6	116	Fair.
9-11	do	do	40 pounds, plus H_2SO_4, 2 pounds	22.3		5.1	115	Poor.
9-13	do	do	80 pounds, plus H_2SO_4, 2 pounds	23.1		4.8	139	Do.
9-15	do	do	120 pounds, plus H_2SO_4, 2 pounds	26.6		4.5	137	Fair.
9-16	do	Sodium chloride	10 pounds	21.1		5.5	170	Poor.
9-17	do	do	20 pounds	23.3		5.0	185	Do.
7, 8-0	Alfalfa (2)	Phosphorus pentasulfide	135 grams	23.7	19.5	4.9	102	Do.
6-3	Alfalfa and canary grass (1-1)	None		28.8	9.6	4.4	190	Good.
6-4	Alfalfa and quackgrass (1-1)	do		29.5	14.2	4.9	156	Poor.
6-21	Alfalfa and timothy (1-1)	do		34.0		4.3	169	Good.
6-22	Clover and timothy (2), (1-1)	do		23.6	13.0	4.5	207	Fair.
8-18	Alfalfa hay and pea vines (1-8)	Molasses	30 pounds	19.6		4.1	90	Good.
8-16	Pea vines	do	30 pounds	18.6		4.1	80	Do.
8-17	do	do	60 pounds	19.4		3.9	84	Do.
6, 9-22	Soybeans (2)	None		24.8	22.0	5.4	126	Poor.
6-5	Soybeans	A. I. V		25.4	7.4	4.0	112	Good.
9-21	do	Molasses	40 pounds	30.6		5.2	127	Poor.
9-20	do	do	60 pounds	30.7		5.1	124	Do.
9-19	do	do	80 pounds	31.3		5.0	118	Do.
6, 9-18	Soybeans (2)	do	100 pounds	28.6	7.8	4.4	112	Good.

SILO LOTS

Small lots of from 5 to 10 tons were put up in silos, two to six different treatments in a silo. Each lot was marked by wooden strips. Table 3 summarizes the analytical data on these silages.

TABLE 3.—*Effect of various preservatives on the composition and quality of silage in silo experiments*

Year	Material	Treatment	Preservative added per ton	Dry-matter content	NH_3–N as proportion of total N	pH value	Carotene per gram of dry matter	Quality, appearance, and odor of silage	Order of palatability [1]
			Pounds	*Percent*	*Percent*		γ		
1938	Alfalfa	Falavigna method		26.3		5.6	19	Poor	
	do	Fresh as ensiled		27.9			105		
1939	Sudan grass	Molasses		26.4		4.2	135	Good	
	do	Fresh as ensiled		30.6			137		
1939	Alfalfa	Whey powder	30	25.5		4.4	126	Fairly good	2
1939	do	Wilted, 40 percent dry matter		42.3	8.2	4.6	90	Poor	5
1939	do	Corn meal	150	27.8	8.2	4.5	121	Fairly good	1
1939	do	None		23.5	17.8	5.3	192	Poor	4
1939	do	Sodium chloride	5	25.3	10.4	4.3	185	Good	3
1939	do	Fresh as ensiled		25.0			200		
1935 to 1937	do	A. I. V. (*14*)		24.5	4.4	3.6	212	Good	(2)
1935 to 1940	do	Molasses (*15, 16*)	65	25.8	7.5	4.1	145	do	(2)
1937 to 1940	do	Phosphoric acid (*16*)	8.3	25.3	9.7	4.3	130	do	(2)

[1] Nos. 1 and 2 were very palatable and distinctly superior to the others, No. 5 was not liked by the animals. The tests were based on the time required to consume the silage at each feeding. The most palatable silage was completely consumed in half an hour, while the less palatable silages were consumed only after several hours.

[2] These silages were all of good palatability.

EXPERIMENTAL RESULTS

NO PRESERVATIVE ADDED

The various legume forages, when put up with no preservative added, gave variable results (tables 1, 2, 3). The bottle silages were mostly poor. The best samples from the barrel silages were those that had been very well packed. In 1939 the packing was not so well done and much poorer silage was obtained. Although the alfalfa with no preservative was well down in the silo with 20 tons of other silage on top of it, still it was poor. While it is possible on occasion to get good silage from fresh material without added preservative, the procedure is not to be recommended.

Cooper (*6*) found that spoilage almost invariably resulted when green alfalfa was put up without added preservative. This has also been reported by Swanson and Tague (*34*), Schieblich (*31*), Newlander et al. (*24*), Politi (*26*), and others. Lavenir and Chaudet (*19*) state that alfalfa put up with no preservative gives variable results, but even in the "good" silage they found 75 percent of the nitrogen in the form of water-soluble compounds and almost entirely amino nitrogen.

On the other hand Neidig and Snyder (*23*) found that sweetclover (cf. table 1) gave silage of excellent quality without the use of a preservatives. Huffman (*15*) summarizes the results as follows:

> Some recent investigations indicate that good legume silage can be made without any additions, and that exclusion of air is the main factor to be considered.

PRESERVATIVES ADDED

Acids and Molasses

The A. I. V. and phosphoric acid procedures gave generally good results. The molasses silages improved with increasing amounts of molasses up to approximately 3 percent for alfalfa and 5 percent for soybeans. No difference was found between the use of cane and of beet molasses, nor between diluted and undiluted molasses as preservatives. All gave uniformly good results. These silages will not be considered further in this paper, as more complete data on other lots are given elsewhere (*14*, *16*).

Whey Powder

Bottle silage treated with powdered whey at the rate of 80 pounds per ton was good, but 20 pounds per ton was not enough. Barrel silage prepared with whey powder at 40 pounds per ton was good, but that with 80 pounds was better. In the silo 30 pounds gave fair results. Thus it seems that 30–40 pounds of whey powder per ton are required to make good alfalfa silage.

Soured-Whey Concentrate

Soured-whey concentrate was prepared as follows: 3,600 cc. of whey was incubated for 48 hours with *Lactobacillus bulgaricus*, at which time it had an acid content of 0.74 percent calculated as lactic acid. This solution was concentrated under vacuum to 600 cc. and a lactic acid content of 4.44 percent. This concentrate was added at the rate of 35, 70, and 140 gm. per kilogram of forage, which corresponds to 420, 840 and 1,680 pounds of liquid whey per ton. (Whey powder at 40 pounds per ton corresponds approximately to

660 pounds of unconcentrated whey per ton.) All samples of soured whey produced good silage, and there was a slight improvement in quality as the amount was increased.

Samarani in 1913 (*29*) proposed the addition of dilute lactose solution as a fermentable carbohydrate in the preparation of grass silage. Whey has been proposed by a number of workers (*1*, *9*, *28*, *39*), but has not given promising results because of its low sugar content. However, very small amounts of whey, of the order of 200 pounds per ton, were used. Wildt (*39*) found no advantage in using whey soured by *Streptococcus lactis*. Allen, Watson, and Ferguson (*1*) obtained good silage with whey added at the rate of 150 gallons per ton (1,360 pounds per ton) and with dried whey added so as to supply 1 pound of lactose per 100 pounds of green forage (about 28 pounds of whey powder per ton). In the case of the dried whey, they found it difficult to obtain even distribution; no such difficulty was experienced in the work reported here.

Corn Meal

Bottle silages prepared with amounts of corn meal from 30 to over 100 pounds per ton (table 1) were poor, and with 120 pounds were only fair. The same result was found with the barrel lots (table 2). The addition of 0.1 percent of sulfuric acid (calculated on the basis of fresh forage) improved the silage somewhat, especially as to the preservation of carotene. In the silo lot, 150 pounds of corn meal per ton gave fairly good silage, but the pH value was still slightly high and the carotene preservation was rather low. However, this silage was very palatable and seemed to show that good alfalfa silage can be made if 150 pounds per ton or over are used.

Swanson and Tague in 1917–18 (*34*) reported good silage with 200 pounds of corn meal per ton of fresh forage. They also tried germinated corn and found that it produced better results than ungerminated corn.

Sulfamic Acid

Sulfamic acid was used in the preparation of some bottle silages, and 50 to 160 pounds of 2.5 N acid per ton gave well-preserved silage. However, it always had a peculiar and rather disagreeable odor.

Soured Molasses

Clover-timothy forage was put up in bottles with molasses that had been diluted 1–3 and soured with *Lactobacillus delbrückii* to pH 4.07 and a lactic acid content of 2.1 percent. This material was added at the rate of 180 pounds diluted or 60 pounds undiluted molasses per ton and gave good preservation and good silage. It was somewhat better than that put up with unsoured molasses.

Salt

Barrel silages were put up with 0.5 and 1.0 percent of sodium chloride, i. e., 10 and 20 pounds per ton, but were poorly preserved in both cases. On the other hand, the silage made in the silo with 5 pounds sodium chloride per ton was well preserved but only fairly palatable (table 3).

Wyant in 1920 (*44*) tried the addition of 1 percent of salt to alfalfa and obtained fairly good silage. Kuckler and Wachter (*18*) used 0.5 percent of salt and 0.5 percent of sugar with good results, while Luchetti (*20*) states that even 0.1 percent of sodium chloride favors the development of acidity. Virtanen (*38*) states that some salt is generally added to the fodder in making ordinary (as distinct from A. I. V.) silage in Finland. The usual amount is from 0.5 to 1 percent of sodium chloride. Analyses are given by him for 98 such samples. Practically all were of poor quality with pH values above 4.6 and ammonia nitrogen amounting to more than 15 percent of the total nitrogen.

Phosphorus Pentasulfide

The phosphorus pentasulfide was burned in the barrel until the barrel was filled with fumes, and then the chopped alfalfa was packed into the barrel. The results were poor, especially as regards carotene preservation (table 2).

SOME OTHER METHODS OF ENSILING

Use of Wilted Forage

Barrel lots were put up with alfalfa that had been wilted to varying degrees, from approximately 27 to 48 percent dry matter. From table 2 it can be seen that poor results were obtained when no preservative was added. The best results were with 46 and 48 percent dry matter. With added molasses good silage was again obtained, but there was no advantage over the unwilted alfalfa. There were disadvantages in wilting, e. g., loss of nutrients, carotene, etc. In the silo the alfalfa that was wilted to 40 percent dry matter gave a very moldy and unpalatable silage, which may have been due to the fact that wilted dried alfalfa does not pack well (*17*, *25*). However, this lot of silage was down in the silo below 10 tons of good silage. Also, as seen in table 3, this silage had a low carotene content. This method of ensiling does not seem advisable as a practical farm procedure.

Cooper (*6*) in 1917 stated that good legume silage could be made if the material was very dry, finely cut, and tightly packed. Eckles (*7*) found that 40 percent dry matter permitted good preservation, while Schieblich (*31*) found it possible to produce excellent alfalfa silage by first drying to 35 percent dry matter. At 15 to 21 percent dry matter, good silage was not obtained unless preservative was added. Bender and others (*2*) found that material high in dry matter (45 to 67 percent) showed considerable charring or molding, while that with 25 to 40 percent dry matter did not; with lower percentages of dry matter putrefaction took place. The high temperature of low-moisture silage could be reduced if sufficient pressure was developed to exclude the air. Similar results as to the possibility of producing silage by partial drying have been reported by many workers (*8*, *13*, *24*, *25*, *32*, *33*, *34*, *39*, *42*, *43*).

Mikhin et al. (*21*) found that green silage with 45 to 50 percent dry matter developed only one-half to one-third the acidity of normal silage. Shepherd and Woodward (*32*, *41*, *42*) have reported good silages with dry-matter content from 30 to 77 percent and pH values from 4.7 to well over 5.0. The writers have found that good silage (as to palatability, odor, appearance, etc.) always has a pH value

well below 4.5. Nehring (*22*) stated that at pH 4.25 or lower the silage was good and free from butyric acid, at values from pH 4.5 to 4.7 the silage was poor, and above pH 4.8 butyric acid was always present and the silage was spoiled. Brouwer (*4*) reports that silage with a pH value above 4.5 is poor, from 4.5 to 4.0 fairly good, and from 4.0 to 3.5 good; and that for practical purposes, a simple examination of pH, odor, and appearance is sufficient to distinguish good silage.

Taylor (*35*) reports, as do Shepherd and Woodward (*32*, *42*), that wilting of the green material prior to ensiling reduced the carotene content to a marked degree. Ellenberger and others (*8*, *36*) state that while grasses or legumes will make good silage without further addition if the moisture conditions are just right, under ordinary farm conditions it is better to use molasses.

A method of storing partly dried forage in silos which is used in Italy has been described by Samarani (*30*) and by others (*5*, *11*, *12*, *26*). Fred and Peterson (*40*) tested this method of ensiling alfalfa. The material was dried to 55 to 70 percent dry matter, then tightly packed into the silo and heavily weighted. The silage produced was brownish in color, of pleasant odor, and was very readily eaten by the cattle. Carbone (*5*) states that this "Crema" silage is more palatable than A. I. V. silage.

Falavigna Method

Falavigna (*10*) in Italy has advocated a method of making silage the essential feature of which seems to be ensiling at a temperature of 50° to 75° C. The silo is filled at such a rate that the forage reaches and maintains this temperature. The material is put in, one layer at a time, each layer being about 1.5 m. deep. Only when the temperature has reached 50° to 75° is another layer added. During the filling, salt is added at the rate of 2.8 pounds per ton. When the filling is completed, the forage is weighted down with stones, earth, or other loading material equivalent to 500 to 1,000 kg. per square meter. It is claimed that under these conditions lactic acid is the main product of fermentation, and butyric acid organisms are excluded by the high temperature.

A temporary silo, made of snow fencing and lined with Sisalcraft paper, was filled with unchopped alfalfa by this method. The temperature during the first week was 50° to 62° C. The analyses are given in table 3. The silage was low in acidity and conspicuously so in carotene, rather dark in color, and of a somewhat disagreeable odor. The palatability was only fair. There was considerable spoilage around the outside of the silo.

Mixed Silages

Mixed silages consisting of alfalfa plus various grasses were put up. The results are given in tables 1 and 2. The silages were only fair, and it would seem advisable to add molasses at about 30 pounds per ton in the preparation of these silages, as was done in the case of the pea vine-alfalfa silage.

The oat-pea bottle silage was put up while the forage was green and succulent, as can be seen from the dry-matter content (table 1), and made a product of very high carotene content.

Soybean silage is seen (table 2) to require more molasses than alfalfa silage. One hundred pounds per ton seemed to be satisfactory in both barrel and silo experiments.

SUMMARY

Over a hundred lots of silage put up in many different ways over a period of several years have been compared as to appearance, odor, preservation, pH value, and carotene content.

Methods considered for the preservation of legume silages included, besides the standard A. I. V., molasses, and phosphoric acid methods, the use of such preservatives as whey powder, soured-whey concentrates, corn meal, sulfamic acid, soured molasses, sodium chloride and phosphorus pentasulfide. Of the latter, whey powder, soured-whey concentrate and corn meal gave the best results.

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THE REDUCING-SUBSTANCE AND PHENOLIC-COMPOUND CONTENT OF THE POTATO TUBER IN RELATION TO DISCOLORATION AFTER COOKING [1]

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INTRODUCTION

In previous publications (*12*, *8*)[2] evidence was presented as to the relative instability of protein in potatoes which darken after boiling. It was found, moreover, (*9*)[3] that tyrosinase activity is considerably greater in tubers which discolor than in those which remain white after cooking. The presence of an activator or of activators of the oxidation of tyrosine in such abnormal potatoes was discovered. Although concentrated tuber extracts gave strong tests for the presence of dihydroxyphenylalanine or other carriers of the catechol structure, it was not possible to characterize any such compound. There was reason for supposing that failure in the available supply of one or more mineral elements was responsible for the metabolic irregularities mentioned above. However, a somewhat extensive examination (*11*) of the mineral content of tubers did not show a correlation between mineral deficiencies and blackening after cooking.

Szent-Györgyi (*10*) has attributed to quinones derived from the catechol structure the capacity to combine with proteins and amino acids to form brown to black pigments, called melanoids. This author supposes that the related oxidizing enzyme, polyphenolase, is rendered inactive in the normal cell and that its activity is released by injury, such as mechanical rupture.

On the other hand, Kertesz (*3*) found that lack of darkening in the Sunbeam variety of peach on exposure of its sliced tissue to the air is not due either to absence or to low activity of oxidizing enzymes. This peculiar difference of Sunbeam from other varieties was found to depend upon deficiency of catechol-tannin compounds. Reference is made by this author to the work of Nightingale, Addoms, and Blake (*5*), which showed that Elberta peaches produced on a low nitrogen supply were much higher in tannin than those from "high nitrogen trees."

EXPERIMENTAL METHODS AND RESULTS

The work reported herein was directed toward more extensive determination of some of the reducing properties of tuber tissue than had been made in the preliminary examination (*11*, *12*). It appeared desirable also, in this connection, to make a somewhat extended com-

[1] Received for publication August 9, 1940. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. The experimental work reported in this paper was supported in part by a grant from the Wisconsin Alumni Research Foundation and was done under the auspices of the University of Wisconsin Works Progress Administration Natural Science Research Program.

[2] Italic numbers in parentheses refer to Literature Cited, p. 358.

[3] ROSS, A. FRANK. THE RELATION OF CERTAIN NITROGENOUS CONSTITUENTS AND ENZYMES OF THE POTATO TUBER TO BLACKENING AFTER COOKING. 1937. [Doctor's thesis, Wis. Univ.]

parison of the catechol content, or its equivalent, and the discoloration record of potatoes.

In the preparatory phases of this investigation relatively unrefined procedure was applied to the measurement of discoloration. The half tubers produced by longitudinal bisection after paring in an abrasive machine were dropped into cold water and boiled until they were readily penetrated by a spatula. They were then removed to a sheet of filter paper, flat face upward, and examined after about half an hour under a fluorescent lamp in a compartment painted black internally. This appears to be a crude method of measurement, but attempts to make use of direct, ocular reflectometer readings on the mashed tissue, packed to a flat surface, were unsatisfactory. The results obtained by this latter method did not give the differences found by direct observation of both localized and diffused blackening of the unmashed potatoes.

THE TOTAL REDUCING CAPACITY OF TUBER TISSUE

Old and New Potatoes

The determination of the total reducing capacity of tuber tissue was the most general of several determinations made for comparison with the contemporaneous color record of boiled potatoes. In most of the work the tubers were pared before boiling, but in the earlier experiments they were boiled in the skins and peeled before inspection for discoloration. It was found that the flesh of cooked sections of unpared tubers, examined when resting upon the epidermis on filter paper, appeared about one grade darker—e. g., dark instead of medium gray—than they did after the epidermis was removed. The total content of reducing substances was determined by the method of Pett (*6*). In this procedure a tissue sample of appropriate weight is ground in a 3.0-percent solution of sulfosalicylic acid, filtered rapidly on a Büchner funnel, washed twice with small volumes of water, and titrated with 0.01 molar potassium iodate, using starch as an indicator.

A preliminary examination was made of 14 samples, covering 6 varieties of potatoes. These were produced mostly in Wisconsin and had been in storage at about 4.5° C. (40° F.) from November 1938 to July 1939. The boiling tests gave results essentially similar to those obtained in December. Values ranging from the equivalent of 15.7 to 21.4 mg. of ascorbic acid per 100 gm. of unpared tuber tissue were obtained, the order of reducing capacity bearing no relation to the degree of discoloration. The ascorbic acid content of these samples was also determined by the current procedure of titrating with dichlorophenol-indophenol. This specific reducing factor ranged in content from 6.3 to 10.8 mg. per 100 gm. of tissue, likewise irrespective of the variation of color from white to medium gray after boiling.

New potatoes of the Triumph and White Rose varieties purchased on the market at this time as received from California and Oregon each contained, within the limits of error, 23 mg. of ascorbic acid and 32 milligram-equivalents of this factor as total reducing capacity. Only a small proportion of the discrepancy between old and new potatoes as to content of ascorbic acid could be accounted for by reversible oxidation, because the stored tubers gave increases of only 1 to 3 mg. on reducing with hydrogen sulfide and removing the residue

of this gas in a current of nitrogen. Due allowance was made for 0.25 cc. of dye solution consumed as the average of several blank determinations of the reduction treatment.

While the results indicate that freshly harvested potatoes may be protected against discoloration, at least in part, by higher levels of ascorbic acid, it does not follow that this abnormality develops in proportion to the destruction of ascorbic acid during fall storage. The potatoes stored for 9 months therefore contained some 60 percent less ascorbic acid and 40 percent less total reducing substances than did the new potatoes examined. It has previously been reported (*12*) that blackening of cooked potatoes is not associated with immaturity of the tubers. The evidence for difference in total reducing power between old and new potatoes as an explanation of discoloration after cooking does not seem to be convincing.

In addition to several samples of Rural New Yorker, a few samples of other varieties were examined for their reducing capacity from 2 to 8 weeks after they were harvested. The tubers were placed in the cold storeroom in the first week of October about 1 week after harvest. Attempts were made to apply selective methods for determining glutathione and ascorbic acid, but these were abandoned in favor of the iodate titration of Pett (*6*). The procedures showed signs of promise, however. In the method of Langou and Marenzi (*4*) for joint determination of ascorbic acid and glutathione by means of Folin's phosphotungstic acid reagent, using cystine for the standard, the greenish color of the unknown as compared with the blue standard renders use of the Duboscq colorimeter infeasible. If proper filters were available, this method might be adapted to the use of the photoelectric colorimeter. The method of Binet and Weller (*2*) for determining glutathione, involving precipitation with cadmium lactate at a specific pH value and titration of the precipitate with potassium iodate, showed some promise of reliability. However, rather than determine individual constituents of questionable significance, it was decided to measure the total reducing level of tissue extracts. The results of this examination appear in table 1.

TABLE 1.—*Relation between the total reducing value and the discoloration after cooking of potato-tubers of 4 varieties taken from cold storage 2 to 4 weeks after harvesting*

Sample No.	Variety	Discoloration after cooking [1]	Original reducing values of raw tissue [2]	Reducing values after reduction by zinc [2]
1	Chippewa	O	23.6	27.6
2	Rural New Yorker	O	28.0	28.0
3		O	27.8	28.2
4		O	29.4	30.4
5		O	24.8	23.6
6		?	21.4	----
7	Sebago	O	23.7	23.3
8		O	25.5	28.0
9	Cobbler	++	24.6	24.0
10		++	26.0	25.0
11	Rural New Yorker	++	18.8	26.0
12		++	17.0	19.0
13		++	20.5	21.2
14		++	17.4	18.0
15		++	20.5	----

[1] O=white; ?=light gray; +=medium gray; ++=dark gray.
[2] Milligram-equivalents of ascorbic acid per 100 gm. of tissue.

In view of the additional data indicative of general absence of reversibly oxidized forms of reducing substances, the determinations on this fraction were discontinued. The direct reducing values for the discolored Rural New Yorker samples were about 40 percent less than for those which were normal after cooking. However, not only the white-cooking Chippewa and Sebago samples but also the discoloring Cobblers showed values falling within the range covered by the better Rural New Yorker samples. Therefore, the results do not support unqualifiedly the concept of protection by reducing substances against discoloration.

As a further test of relationship between total reducing substances and discoloration after cooking, the records of a few samples which showed the common phenomenon of localized discoloration in the stem end of the tubers were examined. As in the preceding determinations, a 5.0-gm. sample was taken from the mixed tissue of several tubers. A record of the results is given in table 2. It will be observed that the data vary extremely from sample to sample. The small difference in some cases in the reducing value of the two ends indicates that much more extensive data would be required to disclose a definite trend, if such exists.

TABLE 2.—*Relation between the total reducing value and the discoloration after cooking of parts of Rural New Yorker potato tubers taken from cold storage 3 weeks after harvesting* [1]

Sample No.	Tuber end	Discoloration after cooking [1]	Reducing value of raw tissue
1	Stem	?	21.4
	Bud	0	18.2
2	Stem	++	19.0
	Bud	+	8.4
3	Stem	++	17.2
	Bud	+	23.5
4	Stem	++	16.6
	Bud	+	14.2

[1] See footnotes to table 1.

The search for relationship between total reducing capacity of the tissue and discoloration after cooking was extended to a few more samples with the results shown in table 3. These data do not show a greater content of reducing substances in the samples which are less subject to discoloration. Their import is supported by the results from a series of Rural New Yorker plantings conducted in cylinders of soil sunk in the field for purposes of control over the supplies of water and mineral nutrients. The crops cooked white throughout but showed a range of from 5.8 to 19.3 mg. of ascorbic acid equivalent as the total reducing capacity. We are thus led to conclude that there is no direct relation between total reducing substances in the raw tuber and whiteness after boiling.

TABLE 3.—*Relation between the total reducing value and the discoloration after cooking of potato tubers of 2 varieties taken from cold storage 5 to 6 weeks after harvesting* [1]

Sample No.	Variety	Discoloration after cooking	Reducing value of raw tissue
1	Rural New Yorker	O	18.3
2		?	17.4
3		?	13.9
4		+	18.3
5		+	19.0
6	Green Mountain	++	15.0
7	Rural New Yorker	++	18.6
8		++	13.7
9		++	17.6
10		++	14.3

[1] See footnotes to table 1.

THE CATECHOL EQUIVALENT OF POTATOES

A series of samples of the Rural New Yorker variety, produced at various locations in Wisconsin was placed at the writers' disposal by Prof. G. H. Rieman of the Wisconsin Agricultural Experiment Station. In view of the activating effect of catechol upon the tyrosinase system (*7, pp. 467–471*) and because of its localization in the cortical region, these samples were subjected to determination of the catechol grouping and to other examination in both the pared and the unpared states. Individual tubers were sectioned longitudinally to provide composite samples. Some of the sections were then analyzed and compared with the boiling record of corresponding sections. The same treatment was applied to other sections of each sample after they had been pared in a small machine of the abrasive type. For the purpose of comparison, the total reducing capacity was determined in the usual manner. The catechol equivalent was determined by treating a portion of the tuber extract according to the procedure of Arnow (*1*). A 2-cc. sample was placed in a colorimeter tube and 2-cc. portions of 0.5 N HCl, 10-percent nitrite-molybdate solution, and 1.0 N NaOH were added successively with shaking. The solution was made to a volume of 10 cc. with water and examined in the Evelyn photoelectric colorimeter with the use of a color filter effective at 5,400 A. The concentration was determined by comparison with a curve prepared by use of standard solutions of catechol. Data from these several determinations are assembled in table 4.

TABLE 4.—*Influence of paring upon the discoloration after cooking, the reducing value, and the catechol content of Rural New Yorker potato tubers grown in different localities and taken from cold storage 8 weeks after harvesting*

Sample No.	Where grown	Discoloration after cooking [1]		Reducing value of raw tissue [2]		Catechol equivalent [3]	
		Pared	Unpared	Pared	Unpared	Pared	Unpared
1	Delavan	O	O	19.2	12.5	2.0	5.8
2	Rice Lake	Od	OD	22.5	24.0	2.7	3.9
3	Starks	++	++d	10.6	18.5	2.4	6.6
4	Antigo	++d	++d	17.4	17.7	3.5	5.2
5	Sturgeon Bay	++d	++d	12.4	16.5	3.4	7.5
7	Rockfield	++	+++	17.1	14.9	3.8	8.2

[1] O=white; ++=medium gray; +++=dark gray; d=mildly discolored cortex; D=seriously discolored cortex.
[2] Milligram-equivalents of ascorbic acid per 100 gm. of tissue.
[3] Milligram per 100 gm. of tissue.

The most prominent feature of the data is the rather consistent increase of catechol equivalent in the pared tuber in relation to blackening after cooking. More data are presented in tables 5 and 6 which support this correlation substantially. It further appears that catechol, or compounds reacting like it, is relatively concentrated in the epidermis and underlying tissues but that discoloration is not directly related to this localization. More data would be required, however, to justify emphasis upon this issue.

Further evidence was sought by comparing tissue from a longitudinal V-shaped section with a portion from a similar section of the same tuber which constituted a thin peel. A sample of Cobbler which cooked light gray contained 6.0 mg. of catechol equivalent in 100 gm. of the total tissue, compared with 13.8 mg. in the peel. Using the plug method, to be described presently, the corresponding values for a Rural New Yorker sample which cooked medium gray were 5.4 and 9.5 mg., respectively.

Additional results which show general correlation between the catechol equivalent and discoloration during cooking, but a partly inverse relation in regard to the total reducing substances, are presented in table 5. The samples here considered were produced from a discoloring seed stock with different fertilizer treatments. As usual, the discoloration bore no consistent relation to any formula of the applied fertilizer.

TABLE 5.—*Relation between the total reducing value and the discoloration after cooking of Rural New Yorker potato tubers taken from cold storage 11 weeks after harvesting*[1]

Sample No.	Discoloration after cooking	Reducing value of raw tissue	Catechol equivalent
1	O	14.5	4.6
2	++	14.9	4.8
3	++	14.6	5.4
4	++	13.3	6.2
5	++D	13.3	5.8
6	++D	11.9	6.2
7	+++	10.5	7.3

[1] See footnotes to table 4.

THE PLUG METHOD OF COMPARING DISCOLORATION WITH REDUCING-SUBSTANCES CONTENT

In view of the concentration of catechol, or compounds reacting as such, in the epidermis or underlying cortical tissue, it became apparent that the tubers should be so sampled as to avoid the effect upon total tissue composition of decrease in proportion of surface as the tubers increased in size. This problem was solved by taking plug or partial core samples from each tuber and using a definite length of such core from the epidermis inward. Five tubers which had been found to discolor seriously by boiling a section of each were pierced longitudinally from the stem end by a No. 8 cork borer. Rapidly, in order to minimize oxidative changes, portions about 1.0 cm. in length were cut from the stem end of these cores to give plugs which bore the epidermis. The plugs were trimmed on their inner ends to give a composite sample weighing 10 gm. This sample was promptly covered

with a 3-percent solution of sulfosalicylic acid and thoroughly crushed in a mortar. It was found necessary to use small-scale filtering equipment (4.25-cm. Büchner funnel and 50-cc. filtering flask) in order to obtain satisfactory agreement of results from duplicate samples. The determinations of total reducing capacity and catechol equivalent were made in the usual manner, but the determination of ascorbic acid was modified by use of an Evelyn photoelectric colorimeter. The results from different varieties and strains thereof as produced on clay loam at Antigo and sandy loam at Hancock in 1939 are shown in table 6. It should be observed that, with the approach of the spring break in dormancy, most varieties could be expected to turn somewhat gray after cooking.

TABLE 6.—*Relation between the discoloration after cooking and certain reducing properties of the tuber tissue of different varieties and strains of potatoes, produced at different locations in 1939, and taken from cold storage 12 to 14 weeks after harvesting*[1]

Sample No.	Variety and strain	Where grown	Discoloration after cooking	Reducing value of raw tissue	Ascorbic acid content	Catechol equivalent
1	Chippewa, A	Antigo	O	12.2	7.6	3.3
2			O	12.6	8.5	3.5
3			O	13.3	8.5	3.5
4			O	13.7	7.8	4.2
5	Cobbler, A		+	14.3	10.6	3.2
6			+	15.2	12.6	3.6
7			++	16.3	12.9	3.6
8			++	16.3	13.5	3.4
9			++	13.8	9.6	5.4
10			++	14.8	11.2	5.3
11			++	15.4	11.4	5.4
12	Rural New Yorker, A		?	15.7	----	3.6
13			++	14.9	----	4.8
14			++	17.9	12.6	5.1
15	Rural New Yorker, B		?d	18.4	14.9	4.9
16			?d	17.7	14.3	4.1
17			+	13.9	10.7	6.0
18			++d	16.8	15.2	5.9
19			++	18.1	16.3	4.5
20	Rural New Yorker, C		?	17.2	10.1	3.9
21			++	13.8	8.6	5.0
22			++	13.5	6.1	5.8
23			++	14.6	8.5	5.4
24	Rural New Yorker, A	Hancock	++d	10.8	----	5.5
25			++	13.0	----	4.5
26			++	12.7	6.1	5.4
27	Rural New Yorker, C		++	5.9	----	5.8
28			++	12.5	6.6	6.8
29			++D	9.5	----	6.3

[1] See footnotes to table 4.

Practically all of the Rural New Yorker samples and all of the Chippewa samples from Antigo were produced with different fertilizer treatments. These included graded levels of potassium supply and the supplementary use of carriers of boron, copper, manganese, and zinc. There is no evidence of correlation between the degree of discoloration and the plane of supply of mineral nutrients. The different strains of Rural New Yorkers are tabulated separately because in the fall boiling test they had given increasing discoloration in the order in which they are recorded. At Hancock the crop came from unfertilized divider rows. This region has the most serious record of discoloring potatoes over a 5-year period in the writers'

experience. Ascorbic acid determinations were purposely omitted in a few cases because of lack of correlation between tuber content of this factor and the record of discoloration in a large number of examinations.

The data show no higher content of total reducing substances in the Chippewa, which cooked white, than in some of the most seriously discoloring samples of Cobbler and Rural New Yorker. Moreover, the predominantly high content of this tissue fraction in the latter varieties as produced at Antigo in contrast to the Rural New Yorker samples from Hancock does not indicate that the general reductive capacity of the tuber tissue has a protective function against pigmentation after cooking.

The ascorbic acid content does not seem to have varied at the time of examination in a manner related to blackening after boiling. In fact, its values show much the same trend as those for total reducing substances. The values for Chippewa are as low as those for some of the most discoloring Rural New Yorker samples.

There appears to be, however, a considerable degree of correlation between the values for catechol and the degree of discoloration. The average value of the catechol equivalent for the different groups of samples is as follows: Chippewa 3.6, Cobbler 4.3, Antigo Rural New Yorker 4.9, Hancock Rural New Yorker 5.7. This record is in accord with the discoloration findings for these varieties and locations over a 5-year period of investigation. In contrast, the average values for ascorbic acid and total reducing capacity are essentially equal for the Cobbler and Rural New Yorker varieties from Antigo and decline markedly in the Hancock crop. It should be noted, however, that the white-cooking Chippewa contained as much catechol equivalent as some of the discoloring Cobblers. The Rural New Yorker shows the most definite correlation between the level of catechol content and the degree of blackening after cooking. Since the tabulated data were obtained, the following results have been secured from the 1940 crop held in cold storage until December or January: Rural New Yorker cooking white, 3.2 and 3.8; Chippewa cooking white, 3.8, light gray, 4.9; Rieman's crosses cooking medium gray, with seriously discolored cortex, 6.5, 6.6, and 8.7.

The present results emphasize the relative abundance of compounds containing the catechol grouping in potatoes that discolor when boiled. Because of the limitations already noted with respect to the evaluation of discoloration, one can hardly expect close correlation between this function and compositional factors. It can be stated with certainty that the compound causing discoloration is related to orthodihydroxyphenol because, in contrast with the pink color of the nitroso derivative here obtained, parahydroxyphenol yields an unstable derivative which turns brown rather promptly, and metahydroxyphenol does not produce a color. This makes it possible that the presence of orthoquinone contributes to pigment formation in accordance with the concept of Szent-Györgyi (*10, p. 68.*)

Spectrophotometric absorption curves of the fraction precipitated by basic lead from alcoholic extracts of discoloring potatoes were found to have nearly the same characteristics as those of catechol and dihydroxyphenylalanine (dopa). The writers found, as had Arnow (*1*) in attempting to determine tyrosine and dihydroxyphenylanine

in a common solution, that the characteristic wave lengths and the breadths of the curve maxima are too similar to permit use of this property for identification. Therefore, we are attempting to isolate derivatives of the compound or compounds involved, for purposes of characterization and identification.

SUMMARY

Earlier work of this laboratory disclosing the presence of an activator of tyrosinase in potatoes which blacken after cooking has been reviewed. Extracts of such disclosing tubers were found to contain an orthodihydroxyphenolic compound, or compounds, resistant to isolation.

Tubers of several varieties from the Wisconsin crop which had been stored at about 4.5° C. for 9 months were about 60 percent lower in ascorbic acid content and 40 percent lower in total reducing substances than Triumph and White Rose potatoes recently shipped to local markets from the West Coast. These were found to contain little reversibly oxidized ascorbic acid.

After cold storage for 2 to 4 weeks after harvest, Rural New Yorker potatoes which discolored contained about 40 percent less total reducing substances (titratable by KIO_3) than did those which cooked normally white. In comparison with the composition of other varieties from the same storage the results do not indicate a protective effect of the total reducing function against the blackening response. Examination for the accumulation of reversible products of oxidation gave generally negative results.

A limited comparison of the total reducing values of tissue from the stem and bud ends of tubers which had been indexed for serious discoloration did not indicate that localized darkening commonly observed in the former region could be ascribed to deficiency of reducing substances.

A comparison of mildly with seriously discoloring Rural New Yorker samples after cold storage for 5 to 6 weeks showed no difference in the range of reducing capacity. An examination of ascorbic acid content in crops of Rural New Yorker produced under different cultural conditions showed that low levels of this constituent were not related to blackening after cooking.

Rural New Yorker potatoes in cold storage for 8 weeks after harvest showed marked concentration in the epidermis and cortex (peel) of a substance or substances giving the catechol reaction. The total reducing substances were not distributed in this manner. After storage for 11 weeks this variety was found to contain amounts of catechol which correlated well with the degree of blackening after boiling, while a somewhat inverse relation existed with regard to total reducing substances.

A method of sampling the tuber tissue (core taking) was adopted to limit the variation between epidermis-cortex and the parenchyma tissue. About 29 samples of three varieties, including the Rural New Yorker from two regions, were examined by this procedure 12 to 14 weeks after they had been placed in cold storage. Higher content of total reducing substances was not correlated with freedom from discoloration after cooking, nor was higher ascorbic acid content.

The content of compounds reacting like, and expressed as the equivalent of, catechol increased from sample to sample somewhat in proportion to blackening after boiling.

In view of the inapplicability of spectrophotometric absorption curves for identifying the orthodihydroxy compound, or compounds, apparently associated with the blackening of boiled potatoes, methods of isolation, and chemical characterization are being applied.

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PHYSIOLOGIC STUDIES OF RHIZOBIUM MELILOTI, WITH SPECIAL REFERENCE TO THE EFFECTIVENESS OF STRAINS ISOLATED IN KANSAS [1]

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INTRODUCTION

Investigations conducted at the Kansas Agricultural Experiment Station have revealed remarkable losses in the nitrogen content of Kansas soils under cultivation. Certain data presented by Swanson and Latshaw [2] indicate that losses in the soil's store of nitrogen may take place even under continuous cropping to alfalfa. Since alfalfa is generally recognized as one of the most efficient nitrogen-gathering members of the Leguminosae, the data presented by Swanson and Latshaw suggest either the absence of adequate numbers of the alfalfa-sweetclover group (*Rhizobium meliloti* Dangeard) of rhizobia in Kansas soils or the presence of relatively low nitrogen-fixing strains. In view of the role that alfalfa and sweetclover play in the soil improvement program in Kansas it seemed worth while to obtain information relative to the distribution and nitrogen-fixing efficiency of Kansas strains of rhizobia capable of infecting these two crops.

A preliminary survey in the vicinity of Manhattan indicated an abundant soil flora capable of producing nodules on alfalfa and sweetclover, provided soil conditions were favorable. This apparent abundance of *Rhizobium meliloti* suggested that if the rhizobia were in any way at fault in the nitrogen-fixing phenomenon, it was probably due to the presence of strains with relatively low nitrogen-fixing capacity.

METHODS AND MATERIALS

ISOLATION OF CULTURES

Nodules were obtained from cultivated alfalfa (*Medicago sativa* L.) plants and from sweetclover (*Melilotus alba* Desr.) plants found along roadsides. Selected nodules were washed in water, immersed approximately 5 minutes in a 1–500 solution of bichloride of mercury, and rinsed in sterile water. Poured plates were then prepared with nitrate-mannitol agar or the medium proposed by Albrecht and McCalla.[3] After incubation for several days at 28° to 30° C., characteristic colonies were selected, tested for purity, and the cultures were kept on the Albrecht-McCalla medium.

TESTING THE INFECTIVENESS AND EFFICIENCY OF CULTURES

Six hundred grams of washed general-run Ottawa sand, mixed with a little calcium carbonate, was introduced into 65 by 500 mm. pyrex test tubes. One hundred thirty-five milliliters of modified Crone's

[1] Received for publication March 18, 1940. Abstract of an unpublished thesis submitted by the senior author to the faculty of Kansas State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of doctor of philosophy. Contribution No. 184, Department of Bacteriology, Kansas Agricultural Experiment Station.

[2] SWANSON, C. O., and LATSHAW, W. L. EFFECT OF ALFALFA ON THE FERTILITY ELEMENTS OF THE SOIL IN COMPARISON WITH GRAIN CROPS. Soil Sci. 8: 1–39, illus. 1919.

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solution,[4] or 15 milliliters less than the amount retained against gravity by the sand, was added. This volume of liquid plus the 15 milliliters of inoculum gave satisfactory moisture conditions. The tubes were plugged with cotton and sterilized at 20 pounds pressure for 1½ hours.

In experiment No. 7 milk bottles were substituted for the pyrex tubes, and approximately 50 percent longer was required to obtain an equal amount of growth; otherwise the results were apparently comparable. Decreased growth in milk bottles was probably due to less adequate lighting.

Alfalfa seeds were shaken for 15 minutes in a 1–500 solution of bichloride of mercury, washed six or more times in sterile distilled water, and germinated on nutrient-agar plates at 28° to 30° C. Eight seedlings were introduced into each tube and the sand medium was inoculated with 15 milliliters of a heavy suspension of cells prepared from young agar slant cultures.

The tubes were then taken to the greenhouse where the plants were allowed to grow for about 7 weeks. If the greenhouse temperature could not be kept low enough to permit good growth of alfalfa, the cultures were transferred to a partially shaded position outdoors where the tubes were buried in the soil to the depth of the sand, and protected from rain by inverting a small tin can over each tube. The volume of liquid held by the 600 gm. of sand was adequate to supply the needs of the plants during the entire growing period; hence, it was not necessary to open the tubes following inoculation.

The following facts would indicate that the conditions within this enclosed system were satisfactory for the object in view: (1) Vigorous growth of the alfalfa plants invariably occurred when inoculated with an effective culture; (2) in no instance did a single plant fail to show one or more nodules when an infective culture was employed as the inoculum; (3) in no instance did an uninoculated plant develop a nodule; (4) the number of nodules developed per plant averaged in excess of 20 in case of highly infective strains; (5) plants invariably made poor growth and exhibited typical nitrogen-hunger symptoms unless inoculated.

Burton and Wilson [5] obtained evidence that the fixation of nitrogen by a strain of *Rhizobium meliloti* may vary with the variety of host plant. Since about 90 percent of the alfalfa grown in Kansas is Kansas Common (*Medicago sativa*), this variety was employed in all tests.

After a growing period of approximately 7 weeks, the plants and sand were emptied onto a sieve from which the sand could be washed readily. The number of plants and nodules in each tube was recorded, and all plant material was oven-dried at 110° C. and analyzed for total nitrogen by the Gunning method.[6] In view of the large amount of sand per tube no attempt was made to recover the nitrogen that may have been excreted into the medium, and it is possible that some nitrogen may have been lost in this way.[7]

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EXPERIMENTAL DATA AND DISCUSSION

In measuring the physiological efficiency of a series of unknowns it is obviously desirable to have some standard with which to make comparisons. There are no standard methods of testing the efficiency or ascertaining the quantities of nitrogen that must be fixed by a given culture of rhizobia before it can be rated as an efficient, or "good," strain. In fact, there are few records of performance available. Under such conditions the most logical procedure appeared to be to obtain from other laboratories cultures rated as efficient and make use of certain of these for comparative purposes. Accordingly, a number of strains of *Rhizobium meliloti* were obtained from research and commercial laboratories, two of the most efficient were selected, and one or both were included in every series of tests conducted. Both strains gave consistently good nodulation, good growth of plant, and somewhat better fixation of nitrogen than the average of locally isolated cultures; hence, served satisfactorily for comparative purposes. In subsequent discussions these two strains, No. 107 from the University of Wisconsin, and A from the Urbana (Ill.) Laboratories, will arbitrarily be regarded as efficient strains, though the limitations in their use as a basis for grading the relative merits of other strains should be kept in mind.

Another fundamental question involved in comparing the relative merits of a series of unknowns is the degree of accuracy of the data upon which the comparison is based. The facilities and time available for conducting such tests were so limited as to restrict the adequate replication of tests for statistical treatment to a very small number of strains. It was believed that a limited study of a large number of strains would give more information relative to the efficiency of the local rhizobia than would a more concentrated study of a few. Consequently each culture isolated was tested once, and when a given strain showed evidence of marked nitrogen-fixing inefficiency it was retested. The retesting of only the apparently inefficient strains was based upon the assumption that the chances for an error in technique being responsible for a combination of good growth and marked accumulation of combined nitrogen, in the absence of fixation by the culture in question, were extremely small. On the other hand, only slight alterations in the environmental conditions might have such an effect upon either of the symbionts as to prohibit nitrogen fixation. This assumption seemed justifiable in view of the fact that the pabulum contained only small quantities of combined nitrogen and all extraneous nitrogen-fixing organisms were eliminated.

In order to secure some concrete information as to the variability in growth, modulation, and nitrogen fixation to be expected in replicated tests of individual strains (*a*) 12 different cultures were replicated 6 times in a single experiment, and (*b*) 1 or both of the 2 cultures chosen for comparison were included in every experiment. In (*a*) the experimental conditions for each of the individual tests of the 12 strains were as nearly identical as it was possible to make them, whereas in (*b*) the 2 strains were subjected to as wide variations as were encountered in the study. It is believed, therefore, that a consideration of the data presented in tables 1 and 2 will give a fair indication of the latitude in growth, modulation, and nitrogen fixation resulting from the experimental error under the experimental conditions employed.

TABLE 1.—*Nodulation, growth, and fixation of nitrogen in the presence of 12 different strains of Rhizobium meliloti*

Strain No.	Plants per culture	Nodules		Dry weight		Nitrogen fixed		Nitrogen content of plants	
		Per plant	Mean [1]	Per plant	Mean	Per plant	Mean [1]	Per group	Mean
	Number	*Number*	*Number*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*	*Percent*
Control	6	0.0		13		0.00		1.38	
	6	0		15	14	0		1.31	1.35
	6	0		14		0		1.35	
A	7	3.6		18		.43		3.31	
	6	2.8		19		.46		3.30	
	6	4.0	4.0±.398	19	19	.45	0.45±.011	3.33	3.36
	5	5.0		21		.48		3.41	
	6	4.7		18		.42		3.43	
4	6	4.2		18		.45		3.39	
	6	4.8		16		.41		3.70	
	7	4.9	4.9±.438	15	18	.36	.45±.037	3.48	3.51
	6	4.0		18		.45		3.48	
	4	6.5		25		.58		3.51	
12–II	6	2.7		17		.43		3.62	
	6	4.2		16		.39		3.68	
	6	3.3	3.7±.160	16	17	.42	.43±.014	3.79	3.68
	6	2.5		18		.47		3.65	
	6	5.8		18		.46		3.65	
18–II	6	6.0		18		.36		2.97	
	6	5.7	6.2±.329	17	17	.38	.36±.015	3.40	3.25
	6	6.8		16		.33		3.39	
27–I	6	7.7		16		.38		3.55	
	6	6.8	8.2±1.020	16	16	.34	.34±.023	3.27	3.30
	6	10.2		16		.30		3.08	
32 III	6	5.0		17		.36		3.21	
	6	12.7		19		.30		2.53	
	6	6.8	8.0±1.340	14	17	.31	.35±.025	3.62	3.22
	6	9.0		19		.43		3.30	
	6	6.5		15		.34		3.45	
54–I	8	4.0		15		.39		3.54	
	6	5.3		16		.31		3.48	
	7	3.9	4.3±.304	15	16	.36	.36±.019	3.49	3.36
	6	3.7		18		.42		3.39	
	6	4.8		18		.34		2.88	
61–I	6	6.3		19		.49		3.49	
	6	4.2		20		.51		3.52	
	6	4.7	4.4±.576	18	19	.47	.47±.018	3.31	3.53
	6	3.2		16		.46		3.94	
	6	2.5		21		.51		3.40	
	6	5.5		17		.40		3.51	
63–I	7	2.6		14		.32		3.39	
	6	3.3		16		.46		3.90	
	6	4.0	3.8±.331	16	17	.35	.42±.027	3.28	3.48
	5	5.0		19		.48		3.59	
	6	4.2		19		.46		3.34	
	6	3.8		18		.44		3.39	
101	6	4.5		17		.48		3.90	
	6	4.5		20		.49		3.32	
	6	3.2	4.2±.325	18	19	.41	.47±.021	3.31	3.47
	6	4.5		21		.51		3.33	
107–I	6	3.5		18		.47		3.71	
	6	4.2		18		.56		4.20	
	6	2.7	3.7±.229	23	19	.54	.52±.016	3.23	3.82
	6	3.8		18		.47		3.73	
	6	4.2		19		.51		3.66	
	6	3.8		17		.54		4.37	
110	6	4.5		16		.43		3.85	
	6	3.7		17		.41		3.46	
	6	3.2	4.3±.425	17	18	.46	.45±.013	3.76	3.50
	6	5.7		20		.47		3.32	
	6	4.5		21		.48		3.13	

[1] Standard error of mean is used.

TABLE 2.—*Nodulation, growth, and fixation of nitrogen in the presence of strains A and 107–I of Rhizobium meliloti*

STRAIN A [1]

Experiment No.	Plants per culture	Nodules per plant	Dry weight per plant	Nitrogen fixed per plant	Nitrogen in plants
	Number	*Number*	*Milligrams*	*Milligrams*	*Percent*
1	7	3.6	18	0.43	3.31
1	6	2.8	19	.46	3.30
1	6	4.0	19	.45	3.33
1	5	5.0	21	.48	3.41
1	6	4.7	18	.42	3.43
3	7	3.7	20	.54	3.39
4	7	5.1	13	.32	3.53
5	6	5.5	16	.41	4.01
6	6	8.0	22	.52	3.08
7	5	5.2	24	.48	2.83
8	5	7.2	23	.47	3.08
9	5	9.6	42	.67	2.20
10	6	8.0	23	.49	2.82
Mean	6	5.6	21	0.47	3.21
Standard error of mean		±0.539	±1.92	±0.024	±0.120

STRAIN 107–I [2]

1	6	3.5	18	0.47	3.41
1	6	4.2	18	.56	4.20
1	6	2.7	23	.54	3.23
1	6	3.8	18	.47	3.73
1	6	4.2	19	.51	3.66
1	6	3.8	17	.54	4.37
2	7	5.0	21	.54	2.99
3	7	5.6	15	.33	3.11
4	6	5.2	13	.38	4.17
5	6	3.8	12	.27	4.19
6	7	4.9	17	.46	3.59
7	6	7.3	22	.38	2.50
8	7	10.3	21	.49	3.18
9	5	9.6	33	.46	2.11
10	5	11.4	30	.69	2.82
Mean	6	5.7	20	0.47	3.42
Standard error of mean		±0.610	±1.466	±0.0266	±0.171

[1] Correlation coefficient between the number of nodules and nitrogen fixed = +0.553; significant at 5-percent level.

[2] Correlation coefficient between the number of nodules and nitrogen fixed = +0.262; not significant at 5-percent level.

Four of the cultures employed in experiment 1 (A, 107, 110, and 101) were received from other laboratories; the remaining eight were isolated locally. Cultures A and 107 were described as "good," 101 as "fair," and 110 as "poor." Preliminary tests had indicated that cultures 4, 18–II, 27–I, 32–III, and 54–I combined in varying degrees the characteristics of inferior nitrogen fixation with relatively high nodule production. On the other hand, cultures 12–II, 61–I, and 63–I had exhibited both good nitrogen fixation and low nodule production.

The data presented in table 1 indicate that four of the locally isolated cultures, i. e., 18–II, 27–I, 32–III, and 54–I, were inferior to the other four and to the four exogenous strains. Three of the inferior local cultures also produced a larger number of nodules. Cultures 4 and 110 apparently had undergone some alteration in nitrogen-fixing ability or perhaps should be classed as "erratic," a term that has been applied to strains exhibiting marked variability in nitrogen fixation.

These data indicate a more marked variability in the number of nodules produced by a given strain than in nitrogen fixed, the latter being rather uniform.

The data in table 1 were subjected to an analysis of variance to determine the significance of differences in the number of nodules formed and the quantities of nitrogen fixed by different strains of rhizobia. The results appear in table 3.

TABLE 3.—*Summary of analysis of variance in nitrogen fixation and nodule formation of strains of Rhizobium meliloti listed in table 1*

Item	Degrees of freedom	Sum of squares	Variance	*F*	5-percent level	1-percent level
Nitrogen fixation:						
Strains of rhizobia	11	0.164	0.0149	6.699	2.00	2.66
Error	46	.103	.0022			
Total	57	0.267				
Level of significance					0.031	0.041
Nodule formation:						
Strains of rhizobia	11	119.076	10.8251	14.080	2.00	2.66
Error	46	35.366	.7688			
Total	57	154.442				
Level of significance					0.568	0.759

If the value of *F* exceeds the values for the 5-percent and 1-percent levels, the odds are 19 to 1 and 99 to 1, respectively, that the variation is, correspondingly, significant and highly significant. If the differences in the mean values for nitrogen fixed and nodules formed, as recorded in table 1, exceed the levels of significance, then the chances are 19 to 1 and 99 to 1, respectively, that such differences are not due to chance.

In other words, the number of nodules formed by strains 18–II, 27–I, and 32–III are significantly higher than the number formed by the other strains, while the quantities of nitrogen fixed by these three strains and also by strains 54–I are significantly lower than for the other strains tested.

An application of the test for significance gave essentially the same results. It is evident from these data that variations in nodule formation and nitrogen fixation can be measured by the method employed in securing the data presented in table 1. It is also believed that these data give a good indication of the degree of variability in nitrogen fixed and nodules formed by a given culture when replicated under as nearly the same conditions as possible. Figure 1 illustrates the remarkable uniformity in growth observed in replicated tests. These data, however, give no indication of the variations that might attend such marked alterations in light and temperature as occurred in subsequent experiments as a result of inability to control these two factors adequately. In order to gain such information, cultures A and 107 were included in every series of tests. The data collected in this manner are recorded in table 2.

As would be expected, variations are to be noted in these data both as regards nitrogen fixed and nodules formed. With few exceptions, however, the values do not vary far from the means. Of the 28 individual tests here recorded of these 2 supposedly good strains, only 1 test fell within the range later to be designated as definitely non-efficient and only 5 fell below the general average for the 217 locally isolated cultures. In 1 test of each culture the quantities of nitrogen fixed were unusually high. Strain 107 exhibited a greater tendency

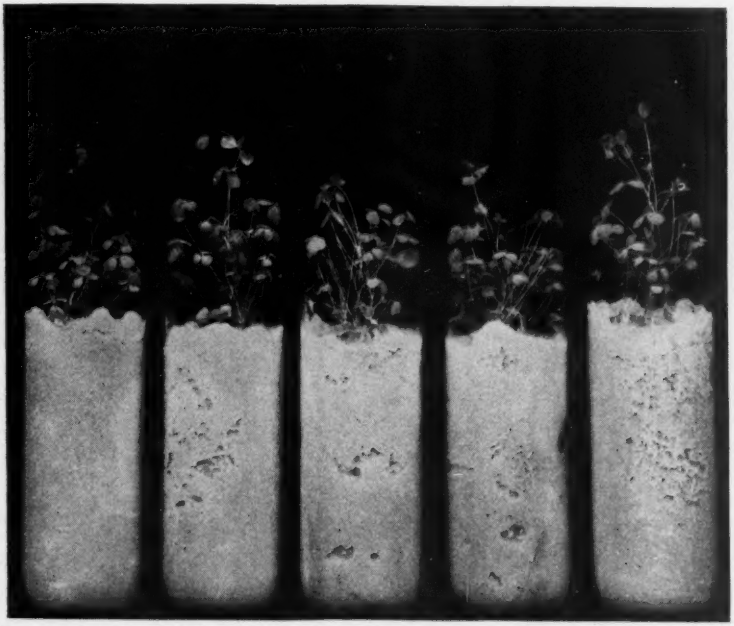

FIGURE 1.—The alfalfa plants in all five tubes were inoculated with the same strain (12–II) of *Rhizobium meliloti*.

to vary both in quantity of nitrogen fixed and in number of nodules formed than did culture A.

The positive correlation between the number of nodules formed and the quantities of nitrogen fixed, a correlation that is definitely significant in the case of strain A, is an interesting fact brought out by the data in table 2. This point will be referred to again.

Altogether 217 cultures of *Rhizobium meliloti* were isolated from alfalfa and sweetclover nodules collected within a radius of 50 miles of Manhattan, Kans. These were tested for their ability to form nodules and fix nitrogen in a series of eight experiments conducted during a period of slightly over a year. A presentation of the complete analytical data accumulated in connection with these tests does not seem essential. The data have been summarized and are presented in tables 4, 5, and 6.

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TABLE 4.—*Nodulation, growth, and nitrogen fixation in the presence of locally isolated cultures of Rhizobium meliloti*

Experiment No.	Cultures tested	Average number of nodules per plant	Average weight per plant	Average nitrogen fixed per plant	Average nitrogen in plant	Correlation coefficient between nitrogen fixed and number of nodules [1]
	Number	*Number*	*Milligrams*	*Milligrams*	*Percent*	
2	45	9.4	23	0.41	2.73	[2]−0.393
3	21	6.5	19	.41	2.93	[3]−.067
4	35	5.8	15	.38	3.73	[3]+.0145
5	19	5.1	15	.37	3.92	[3]+.044
6	16	10.3	18	.34	2.77	[4]−.613
7	36	7.8	21	.34	2.48	[4]−.387
8	41	10.8	22	.45	2.94	[2]−.492
9	4	16.3	12	.32	2.56	[3]+.45
Mean		8.2	19	.39	3.01	

[1] The correlation coefficient between nitrogen fixed and nodules produced for the entire 217 cultures was −0.202, a highly significant value.
[2] Highly significant.
[3] Not significant.
[4] Significant.

TABLE 5.—*Classification of locally isolated cultures of Rhizobium meliloti according to nitrogen-fixing ability*

Experiment No.	Cultures tested	Cultures in indicated range of nitrogen fixation			
		<0.30 milligram	0.30–0.39 milligram	0.40–0.46 milligram	>0.46 milligram
	Number	*Number*	*Number*	*Number*	*Number*
2	45	11	13	8	13
3	21	3	8	3	7
4	35	5	18	6	6
5	19	4	7	7	1
6	16	3	10	2	1
7	36	12	12	1	11
8	41	5	7	10	19
9	4	2	0	1	1
Total	217	45	75	38	59
Proportion of total		*Percent* 20.7	*Percent* 34.6	*Percent* 17.5	*Percent* 27.2

TABLE 6.—*Relationship between number of nodules formed and nitrogen fixed by 217 cultures nf Rhizobium meliloti*

Number of nodules per plant	Cultures	Average number of nodules	Average nitrogen fixed
	Number	*Number*	*Milligram*
Less than 5	48	3.9	0.41
Between 5 and 7.5	64	5.9	.40
Between 7.5 and 10.0	54	8.6	.41
Between 10.0 and 12.5	22	11.0	.43
Between 12.5 and 15.0	10	13.6	.38
Between 15.0 and 20.0	10	17.1	.28
More than 20.0	9	24.2	.26

The average weight, number of nodules, and quantity of nitrogen fixed per plant and the percentage of nitrogen in plants for each experiment and for the entire 217 tests are recorded in table 4. In table 5 is recorded the frequency distribution in nitrogen fixed per plant. The divisions of the data in this latter table are more or less arbitrary.

The mean nitrogen fixed by the 217 cultures was 0.39 mg. and that by the two presumably good strains was 0.47 mg. It is assumed that any culture equaling or exceeding the two control cultures in efficiency should be ranked as "good." It is also arbitrarily assumed that any culture fixing less than 0.30 mg. should be ranked as "poor." Of all cultures examined 55.3 percent were below and 44.7 percent above the mean and are regarded as "inferior" and "superior," respectively; 20.7 percent are classed as "poor" and 27.2 percent as "good."

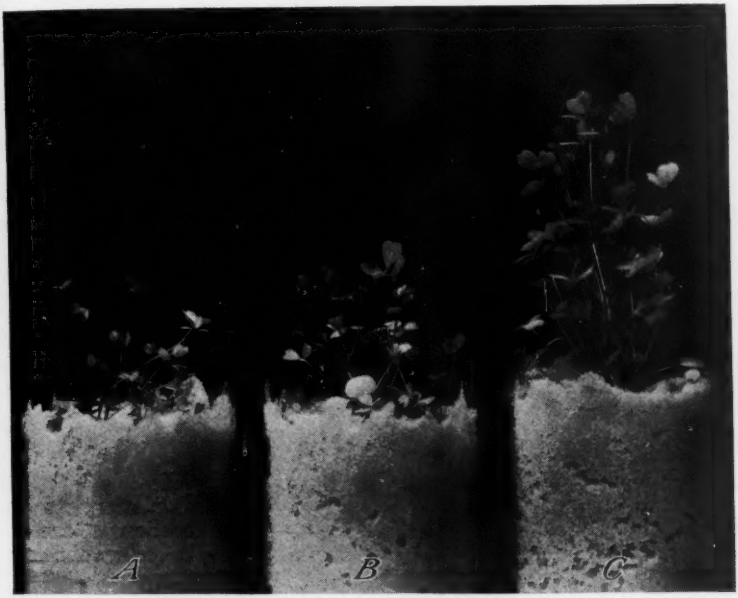


FIGURE 2.—*A*, uninoculated alfalfa plants; *B*, alfalfa plants inoculated with an inefficient strain (192–V) of *Rhizobium meliloti; C*, alfalfa plants inoculated with an efficient strain (A) of *R. meliloti*.

Figure 2 illustrates the difference in growth observed between plants inoculated with "poor" and "good" cultures.

The data presented in table 4 show a surprising variation in the average number of nodules per plant in the different experiments. A careful inspection of the complete data will show, however, that this is accidental in that by chance there were relatively more cultures tested in experiments 6, 8, and 9 that formed a large number of nodules, and hence gave a high average nodule production for those experiments. Three of the four cultures in experiment 9 were high nodule-producing cultures.

Attention has been called by a number of investigators to an apparent negative association between the production of nodules and efficient nitrogen fixation. The data here presented seemed adequate to determine by statistical treatment the presence or absence of such an association. Accordingly, the correlation between these two factors has been calculated for each experiment as well as

for the entire 217 cultures, and the value of r (correlation coefficient) is recorded in the last column of table 4. Applying a test for significance to these r values it is found that in the case of experiments 6 and 7 the correlation is significant, i. e., above the 5-percent level, while for experiments 2 and 8, and for the entire 217 cultures, it is highly significant, i. e., above the 1-percent level. On the other hand, the r values for experiments 3, 4, 5, and 9 are not significant. An analysis of the complete data will reveal a relatively high degree of uniformity in nodule production in experiments 3, 4, and 5, indicating the absence of high nodule-producing cultures; the number of comparisons in experiment 9 is too small to be of any value. In other words, if, in a given series of tests, there happened to be present some cultures of high nodule-producing capacity, a significant negative correlation was found between nodule production and nitrogen fixation. In the absence of (at least a few) high nodule-producing strains no such correlation was found between these two factors.

As further evidence in support of the conclusion reached in the preceding paragraph, the correlation coefficient was calculated between nodules produced and nitrogen fixed by 40 cultures that were retested. These cultures were selected because of wide variation in nitrogen fixation and nodule production and combinations of these two characteristics. The value of r for these 40 pairs of comparisons was -0.523, a highly significant value. It seems safe, therefore, to assume that the cultural or strain characteristics of nodule production and nitrogen fixation are definitely negatively associated.

On the other hand, if a comparison is made between the number of nodules produced and nitrogen fixed by a single given strain, a positive correlation is obtained, as is evident from the data presented in table 2 for cultures A and 107.

The available data are inadequate to establish definitely whether low fixation of nitrogen, in the presence of high nodulation, results from or is associated with some physiological alteration in one or both of the symbionts, or whether high nodule-producing and low nitrogen-fixing ability are associated inherent characteristics.

The fact that of 28 individual tests conducted with the 2 control cultures under a wide variety of conditions not one gave nodulation above the level for efficient nitrogen fixation (12.5–15.0 nodules per plant), and that a positive correlation was evident between nodulation and nitrogen fixation in case of the individual strains, might be interpreted as indicating that both inheritance and some physiological factor were operating. In other words, it is possible that a given efficient culture is inherently limited in its ability to produce nodules, but any environmental factor which may tend to raise nodule production into the upper range of a culture's capabilities will result in increased nitrogen fixation. This point is borne out by experience, since abundant nodulation by a "good" strain is considered more valuable than limited nodulation by the same strain.

It is of interest to note that the number of local cultures producing a large number of nodules was not very great. In table 6 the 217 cultures are arranged into 7 groups according to the number of nodules produced. It appears from these data that the number of nodules gives little indication as to nitrogen-fixing efficiency except as the number may be less than or may exceed 12.5 to 15.0 per plant. It has already been suggested that a negative correlation between

nodulation and nitrogen fixation is not evident unless some high nodule-producing strains are present, and the data in table 6 indicate quite clearly why this is true.

There is also some indication in these data that the larger the number of nodules, provided excessive nodulating strains are not involved, the more nitrogen there will be fixed, in that the 10–12.5 nodule group gave the highest average nitrogen fixation.

The data presented in connection with strain 107, table 2, indicate that considerable variation in nodule production is to be expected in some good strains, and the maximum average nodule production recorded for this strain (11.4) is approaching the point of maximum nodulation compatible with efficient fixation. The indications, therefore, are that variations in nodulation up to about 12.5 nodules per plant, as determined in these experiments, is normal for good strains, while the production of nodules in excess of 15 per plant points to an inherent characteristic of high nodule-forming ability associated with low fixation of nitrogen. An examination of the complete data will show that there are sufficient exceptions to this generalization to indicate that other, as yet unidentified, factors may be operating.

SUMMARY

An enclosed system of growing alfalfa plants for testing the nodule-producing and nitrogen-fixing efficiency of cultures of rhizobia is described. The method is adapted to both greenhouse and outdoor conditions and requires a minimum of space and attention while plants are growing, and, at the same time, affords a maximum of protection against accidental inoculation. Quantitative measurements of growth, nodulation, and nitrogen fixation are easily attained, and replicated tests as well as repeated tests, at least of "stable" cultures, agree well.

Two hundred and seventeen cultures of rhizobia were isolated from alfalfa and sweetclover in the vicinity of Manhattan, Kans., and their nodule-forming, nitrogen-fixing, and growth-stimulating abilities measured quantitatively by the method described. In comparison with exogenous "good" strains, 27.2 percent of these cultures proved equal or superior, hence, may be classed as "good;" while 20.7 percent were sufficiently inferior to the general average to be classed as "poor."

A comparison of the number of nodules formed with the quantities of nitrogen fixed by the 217 cultures revealed a significant negative correlation between these two characteristics. The evidence indicates, however, that only when the number of nodules formed is relatively high, i. e., more than 12.5 to 15.0 per plant in these tests, does high nodulation become incompatible with efficient nitrogen fixation. Extensive studies with two separate "good" strains gave a positive correlation between nodulation and nitrogen fixation, but in no case did nodulation attain the minimum suggested as being associated with inefficient nitrogen fixation. These facts suggest the inherent association of high nodulation with inefficient nitrogen fixation and low nodulation with efficient nitrogen fixation. Further studies along these lines are highly desirable.

FACTORS AFFECTING ONION PUNGENCY[1]

By HANS PLATENIUS, *research assistant professor of vegetable crops*, and J. E. KNOTT, formerly *research professor of vegetable crops, New York (Cornell) Agricultural Experiment Station*

INTRODUCTION

Both mild and pungent onions are demanded for food, the particular use being made of them determining the type required. When onions are used in salads, for seasoning, or as a cooked vegetable, bulbs of relative mildness are usually preferred. On the other hand, a market for highly pungent varieties is created by the manufacturers of such products as sauces, canned soups and extracts, sold in the form of onion powder and onion salt.

The factors influencing the pungency of onions should be of interest to producers and buyers, especially those who use large quantities of this vegetable for manufacturing various food products. Heretofore, no such study has been carried out, mainly because no method was available of measuring small differences in pungency with any degree of accuracy. By means of tasting tests, it is possible to decide whether one particular bulb is stronger than another one, but such a procedure hardly lends itself to an accurate classification of a large number of varieties.

The particular constituent to which the onion (*Allium cepa* L.) owes its pungency was first identified by Lemmler [2] as a volatile oil, known chemically as allyl-propyl-disulfide. Based on these findings, a method was developed by Platenius [3] for determining quantitatively the content of volatile oil in the bulbs. After prolonged hydrolysis, the onion oil is distilled and the total sulfur content is determined in the distillate. The dependability of this method has frequently been checked against tasting tests, and in every instance the differences found noticeable by tasting tests corresponded to differences in the volatile-sulfur content of the bulbs. This fact cannot be considered as conclusive proof of the reliability of the method; still, it is the only confirmation of its value available at the present time.

Preliminary experiments carried out by the writers established the fact that onions differ in pungency not only as a result of genetic factors but also because of soil and other ecological conditions. It was found, for instance, that Italian Red onions imported from Italy were consistently milder than bulbs of the same variety grown in New York. In an earlier publication,[4] the writers also showed that the pungency of onions increases with maturity until the tops begin to fall over. This makes it necessary to compare the pungency of different varieties when the bulbs have reached the same degree of maturity.

[1] Received for publication June 29, 1940. Paper No. 217, Department of Vegetable Crops, Cornell University.

[2] LEMMLER, F. W. DAS ÄTHERISCHE ÖL DER KÜCHENZWIEBEL (ALLIUM CEPA L.). Arch. der Pharm. 230: 443–448. 1892.

[3] PLATENIUS, HANS. A METHOD FOR ESTIMATING THE VOLATILE SULPHUR CONTENT AND PUNGENCY OF ONIONS. Jour. Agr. Res. 51: 847–853, illus. 1935.

[4] PLATENIUS, HANS, and KNOTT, J. E. THE PUNGENCY OF THE ONION BULB AS INFLUENCED BY THE STAGE OF DEVELOPMENT OF THE PLANT. Amer. Soc. Hort. Sci. Proc. 33: 481–483, illus. 1936.

The present study was undertaken to classify the most important commercial varieties with respect to pungency and to ascertain to what extent factors such as soil conditions, temperature, moisture supply, and storage influence the pungency of any one variety.

RELATIVE PUNGENCY OF DIFFERENT ONION VARIETIES

In the study carried out in 1936 and 1937 a comparison was made of the relative pungency of 16 varieties of onions, including most of the commercially important types. As is commonly known, certain varieties, such as the Bermuda, are poorly adapted to production in northern latitudes because of the unfavorable combination of day length and temperature during the growing season. For that reason, six of the varieties studied were obtained from the Texas substation at Winter Haven,[5] while all the others were grown at Ithaca, N. Y.

At Winter Haven, the usual cultural practices of that region were followed. The soil, a fine sandy loam, received an application of 250 pounds of 11–48–0 fertilizer per acre, and the necessary moisture was supplied by furrow irrigation. The onions were grown from seed, and the seedlings were set out in December. The mature bulbs were harvested in April, shipped to Ithaca by express, and analyzed for volatile-sulfur content within a few days after they were received.

At Ithaca, 10 varieties adapted to northern climates were grown on a gravelly sandy loam, which had received an application of 10 tons of manure and 1,500 pounds of a 5–10–5 fertilizer per acre. The onions were started from seed in February, and the seedlings were transplanted to the field the end of April and harvested during August and September. An overhead irrigation system supplied adequate moisture during periods of insufficient rainfall.

Because it is known that the stage of maturity has an influence on the degree of pungency, care was taken to harvest all varieties when they had developed their maximum content of volatile sulfur, or when the tops began to fall over and wilt. Analyses for volatile sulfur were made within 2 weeks after harvest. A composite sample from at least 25 bulbs was taken for analysis. Dry-weight determinations were made on 100-gm. aliquots dried in a ventilated oven at 60° C. for 48 hours.

The results of this study are presented in table 1, which lists the different varieties in ascending order of pungency based on the determinations made in 1937. Because the data for early varieties grown in Texas are hardly comparable with the data for those from New York, the varieties are grouped separately according to their source.

It will be noticed that, in general, the volatile-sulfur content of the onions was higher in 1936 than in 1937, probably because of differences in the prevailing climatic conditions. At Ithaca the growing conditions were more favorable for the development of the bulbs in 1936 than in 1937, as indicated by a considerable difference in the average weight of the bulbs of each variety at harvest time. More important is the fact that the order, or relative pungency, was the same for all of the early Texas varieties in both years, and that in the late varieties this order was changed only slightly in four of the varieties studied.

[5] The writers are indebted to Mr. L. R. Hawthorn of the Texas Experiment Substation No. 19, Winter Haven, Tex., for supplying the samples in 1936 and 1937.

TABLE 1.—*Volatile-sulfur content on a fresh-weight basis, percentage dry matter, and average fresh weight per bulb of 16 varieties of onions grown at Winter Haven, Tex., and at Ithaca, N. Y., in 1936 and 1937*

Variety and where grown	Results for 1936			Results for 1937		
	Average fresh weight per bulb	Volatile-sulfur content	Dry-matter content	Average fresh weight per bulb	Volatile-sulfur content	Dry-matter content
Winter Haven, Tex.:	*Grams*	*P. p. m.*	*Percent*	*Grams*	*P. p. m.*	*Percent*
F. P. I. 106054				165.8	61.1	10.01
Early Grano	210.5	68.1	8.71	206.5	65.2	7.35
Yellow Bermuda	132.6	72.3	10.58	125.9	87.4	8.97
Crystal Wax	134.2	97.9	10.27	106.2	98.4	8.94
White Creole	57.2	129.1	19.20	69.3	127.6	8.63
Red Creole	81.2	155.3	19.40	70.2	143.1	9.69
Ithaca, N. Y.:						
Early Grano	220.1	67.5	6.97	148.7	59.5	7.22
Early Yellow Globe	239.4	94.4	9.53	152.6	72.5	9.82
Utah Sweet Spanish	393.6	97.9	7.89	221.0	78.7	6.87
Red Rocca				205.0	81.1	7.97
Mountain Danvers	306.8	126.6	10.72	143.6	88.2	9.42
Red Wethersfield	268.5	123.0	12.06	146.6	98.3	10.73
Brigham Yellow Globe	265.2	117.3	12.08	176.9	116.2	10.31
Yellow Globe Danvers	244.8	124.5	12.08	151.4	121.5	16.66
Ebenezer	156.0	156.9	14.88	122.0	134.1	13.92
Australian Brown				123.0	144.5	10.13

Early Grano, the only variety grown in both Texas and New York in this experiment, proved to be one of the mildest types in both groups. It was exceeded in mildness only by F. P. I. 106054. Red Rocca, suprisingly, was more pungent than some of the native varieties, although when imported from Italy it is known to be the mildest onion available. This suggests that under certain conditions the influence of soil and climate may be more important than genetic factors in determining the pungency of onions. Early Yellow Globe and the Utah Sweet Spanish are other varieties which, on the basis of these data, must be considered mild. In the medium-pungent groups should be listed the yellow Bermuda, Crystal Wax, Red Wethersfield, Brigham Yellow Globe, and Yellow Globe Danvers. Outstanding as pungent varieties are the Creoles, Ebenezer, and Australian Brown. Obviously these last-named varieties should be given first consideration in selecting stock for manufacturing purposes for which onions of extreme pungency are desired.

From a study of the moisture content of different varieties of onions. Jones and Bisson [6] drew the following conclusion:

> Evidently, those varieties that are considered mild and of rather poor storage quality have the highest moisture content, whereas those that are the most pungent and keep best in storage have the lowest moisture content.

This suggests that pungency in any variety is merely a matter of relative concentration of the dry matter, including onion oil. To some extent, this view is supported by the data in table 1, although it must not be overlooked that there are several notable exceptions. Australian Brown and F. P. I. 106054, the strongest and the mildest varieties included in the study carried out in 1937, both had a medium water content of 90 percent.

[6] JONES, H. A., and BISSON, C. S. MOISTURE CONTENT OF DIFFERENT VARIETIES OF ONIONS. Amer. Soc. Hort. Sci. Proc. 31: 165–168. 1934.

In this connection attention should be called to the fact that in these experiments there was fairly good correlation between the average fresh weight of the bulbs and their relative mildness.

EFFECT OF ENVIRONMENTAL FACTORS ON ONION PUNGENCY

TEMPERATURE

In 1936 an experiment was conducted in the greenhouse to determine the effect of temperature on the pungency of onions. Thirty plants of Yellow Bermuda were grown in each of several iron drums 22 inches in diameter and 17 inches in depth. The drums were filled with a sandy-loam soil; and by means of an automatic watering device, connected by glass tubing to the base of the drum, a water table 12 inches below the surface was maintained. Two drums were placed in each of three greenhouses, in which the respective temperature ranges were from 50° to 60°, 60° to 70°, and 70° to 80° F. The experiments were started in January and the bulbs were harvested in June. Because it was impossible during the last month of growth to maintain an average temperature below 70° in any of the houses, the data from this experiment must be considered as inconclusive. However, since such striking differences were obtained from the temperature treatments, the results of this expreiment are presented in table 2. According to these data, the onions grown at a temperature of from 70° to 80° were three times as pungent as those grown at from 50° to 60°. In evaluating these data, consideration must be given to the physiological age of the plants. At the time the onions were harvested the tops of the plants in the warmest house (70°–80°) had died down completely, whereas those in the coolest house (50° to 60°) were still growing vigorously. Differences in pungency in the three lots can therefore be attributed in part to differences in the stage of maturity. In an earlier paper [7] the writers pointed out that until the time when the tops begin to fall over the pungency of the bulbs increases steadily. Undoubtedly the results of this experiment would have been less striking if the bulbs from the three temperature treatments could have been harvested when the plants had all reached the same physiological age.

An attempt to repeat this experiment in 1937 failed because of serious damage to the plants by red spider and thrips at the high temperature.

TABLE 2.—*Volatile sulfur content on a fresh-weight basis, percentage of dry matter, and average fresh weight per bulb of Yellow Bermuda onions grown in the greenhouse at three different temperatures.*

Growing temperature [1] (°F.)	Average fresh weight per bulb	Volatile-sulfur content	Dry-matter content
	Grams	*P. p. m.*	*Percent*
50°–60°	65	42.8	8.37
60°–70°	74	80.0	7.98
70°–80°	46	130.9	7.38

[1] During the last month of growth, the temperature in all houses varied from 70° to 80°.

[7] See footnote 4.

WATER SUPPLY

Seedlings of Early Grano, Ebenezer, and Utah Sweet Spanish were transplanted to the field late in April 1936. The sandy-loam soil received an application of 10 tons of manure and 1,500 pounds of a 5–10–5 fertilizer per acre. The rows of each variety were so arranged that one-half of each row could be supplied with additional water by overhead irrigation. During the growing season of May 1 to September 1 a total of 9.41 inches of rain fell. One-half of each row received sufficient irrigation to provide in all about 1 inch of water a week, or a total of 17 inches during the growing season of 4 months. When the tops had fallen over, the bulbs were harvested and their content of volatile sulfur determined. The data are presented in table 3. In every instance the bulbs grown with additional water were larger and less pungent than those which had been dependent on the natural rainfall. The reduced pungency of Early Grano and Ebenezer as a result of irrigation was due in part at least to the higher water content of the irrigated bulbs, which tended to reduce the concentration of volatile sulfur in the bulbs when the data were expressed on a fresh-weight basis.

TABLE 3.—*Effect of irrigation on the average weight of bulbs, the content of volatile sulfur on a fresh-weight basis, and the percentage of dry matter of onions of three varieties*

Variety	Water supply	Average fresh weight per bulb	Volatile-sulfur content	Dry-matter content
		Grams	*P. p. m.*	*Percent*
Early Grano	Not irrigated	154.2	82.5	7.35
	Irrigated	220.1	67.5	6.97
Ebenezer	Not irrigated	77.5	162.3	15.42
	Irrigated	156.0	156.9	14.88
Utah Sweet Spanish	Not irrigated	208.4	103.6	7.72
	Irrigated	393.6	97.9	7.89

No doubt the effect of irrigation on the pungency of the bulbs depended on the increase in available soil moisture. If the natural rainfall had been higher during that particular season, differences in pungency between irrigated and nonirrigated lots would probably have been negligible.

SOIL TYPES AND WATER SUPPLY

In the second experiment the influence of soil types and water supply were studied in the greenhouse at an average weekly temperature of 60°–70° F. Iron drums 22 inches in diameter and 17 inches in depth were used as containers for the soil. Four drums were filled with a well-decomposed woody peat, four with a sandy loam, and four with a sandy soil. In two drums of each soil type the water table was maintained 12 inches below the surface; in the remainder it was held at a depth of 16 inches. The water table was controlled by means of an automatic watering device connected by glass tubing to the base of the drum. The low water level provided adequate moisture for the growth of the onions in the peat soil without surface application of water. It was found necessary, however, to add occasionally an equal quantity of water to the surface of each of the drums containing

sandy loam and sandy soil at a low water level in order to maintain growth.

Fertilizer of 5–10–5 analysis was added to each drum at the rate of 1,000 pounds per acre. In October 1936, 30 sets of Yellow Globe Danvers were planted in each drum. Beginning in January 1937 a 15-hour photoperiod was provided by means of Mazda lights. The bulbs were harvested in May 1937, and their content of volatile sulfur was determined. The data in table 4 indicate that the higher content of soil moisture increased the average weight of the bulbs and decreased the volatile-sulfur content when compared with bulbs grown at a low water table. These experiments are in agreement with those obtained outdoors in experiment 1. There was also a marked difference in the pungency of the bulbs, depending on the soil type in which they were grown. The most pungent onions were harvested from the peat, the mildest from the sandy soil.

TABLE 4.—*Effect of soil type and sulfur content and of the water supply on the average weight of bulbs, the content of volatile sulfur on a fresh-weight basis, and the percentage of dry matter of Yellow Globe Danvers onions, grown in the greenhouse*

Soil type	Approximate percentage of sulfur in the soil	Water supply	Average fresh weight per bulb	Volatile-sulfur content	Dry-matter content
	Percent		*Grams*	*P. p. m.*	*Percent*
Peat	0.470	Low	40.4	101.4	12.56
		High	51.8	85.8	12.90
Sandy loam	.039	Low	26.6	80.3	13.19
		High	60.1	74.9	12.84
Sandy soil	.004	Low	41.0	64.0	13.01
		High	50.0	49.5	12.88

The approximate values for the total sulfur content of the three soil types used in this experiment are included in table 4. These data are averages determined for New York soils by Wilson and Staker[8] and by Bizzell.[9] The addition of the equivalent of 1,000 pounds of 5–10–5 fertilizer per acre to the drums did not change these values appreciably.

A striking correlation exists between the pungency of the onions and the soils on which they were grown. However, this relationship is by no means proportional. Whereas the sandy loam contains roughly 10 times and the peat 100 times as much sulfur as the sandy soil, the corresponding volatile-sulfur content of the onions was only 25 and 58 percent higher on the sandy loam and peat than it was on the sandy soil. It should be remembered that the sulfur analyses of the different soil types include both available and unavailable forms of sulfur. These comparisons are based on determinations made for pungency of onions grown with a low water supply. A similar relationship exists between those grown on soils with a high water table.

APPLICATION OF SODIUM SULFATE

Drums similar to those used in the previous experiment were filled in duplicate with a well-decomposed peat, a clay loam, and a sandy loam. Bank sand was placed in four additional drums. All were

[8] WILSON, B. D., and STAKER, E. V. THE CHEMICAL COMPOSITION OF THE MUCK SOILS OF NEW YORK. N. Y. (Cornell) Agr. Expt. Sta. Bul. 537, 26 pp. illus. 1932.

[9] BIZZELL, J. A. THE CHEMICAL COMPOSITION OF NEW YORK SOILS. N. Y. (Cornell) Agr. Expt. Sta. Bul. 513, 25 pp., illus. 1930.

fertilized with an equivalent of 1,000 pounds of a 5–10–5 analysis per acre. In addition, two of the drums containing bank sand received an application of sodium sulfate at the rate of 600 pounds per acre. The water table was maintained automatically at a level of 16 inches below the surface in all drums. Only distilled water was used for irrigation. Yellow Bermuda were transplanted, 30 to a drum, in October 1937; the mature bulbs were harvested in May 1938. The temperature of the greenhouse was maintained at 60° to 70° F. and the daily photoperiod lengthened as in the previous experiment. The data in table 5 again show that onions grown on peat soil are not only larger but also considerably more pungent than those from other soil types. The smallest and mildest onions were harvested from sand and sandy loam soil.

TABLE 5.—*Effect of soil type and of sulfate content of the soil on the average weight of bulbs, the content of volatile sulfur on a fresh-weight basis, and the percentage of dry matter of Yellow Bermuda onions grown in the greenhouse*

Soil type	Approximate percentage of sulfur in the soil	Average fresh weight per bulb	Volatile-sulfur content	Dry-matter content
	Percent	*Grams*	*P. p. m.*	*Percent*
Peat	0.470	63.2	157.2	11.11
Loam	.058	48.2	125.2	10.42
Sandy loam	.039	39.3	86.4	10.58
Sand (low sulfate)	.004	20.4	72.8	8.77
Sand (high sulfate)	.008	30.6	77.6	8.70

Again there was a direct relationship between the pungency of the bulbs and the sulfur content of the soils on which they were grown. The addition of an equivalent of 600 pounds of sodium sulfate per acre to the sandy soil doubled the percentage of sulfur present but was insufficient to have an appreciable effect on the pungency of the onions. At any rate, the slight increase in volatile sulfur of the bulbs from sand to which sulfate had been added is probably not significant.

These results lead to the conclusion that on a commercial scale it is not feasible to increase the pungency of onions by the addition of fertilizers of a high sulfur content because the quantities required to produce a noticeable effect is unreasonably large.

STORAGE

Experiments to study changes in the pungency of onions held in cold storage were undertaken in 1936 and 1937. Yellow Globe Danvers and Utah Sweet Spanish were stored in bushel crates and kept in an experimental cold-storage room at 33° F. Temperature fluctuations in the room did not exceed $\pm 1.5°$. At intervals samples were taken for analysis to determine changes in the content of volatile sulfur and moisture. At the same time, records were taken of the shrinkage that occurred during the different storage periods. Since the bulbs showed neither spouts nor decay none were discarded during the storage period; consequently shrinkage data represent weight losses only.

The results of these experiments are summarized in table 6. It will be noticed that the volatile-sulfur content increased as the storage

period progressed. However, this increase in pungency appeared to proceed at a slower rate toward the end of the storage period; in fact, the lot of Yellow Globe Danvers stored in 1937–38 showed a slight decrease in its content of volatile sulfur.

Shrinkage losses and changes in the percentage of dry matter are partly responsible for variations in the volatile-sulfur content of the different samples. However, table 6 reveals that these two factors alone cannot account for the general tendency of volatile sulfur to increase during the storage period. Instead, it is evident that some of the onion oil was formed during storage from other forms of sulfur and organic constituents in the bulbs.

TABLE 6.—*Shrinkage, content of volatile sulphur on a fresh-weight basis, and percentage of dry matter of onions of two varieties when held in storage at 33° F. for different periods*

Variety	Date sampled	Shrinkage	Volatile-sulfur content	Dry-matter content
		Percent	*P. p. m.*	*Percent*
Yellow Globe Danvers	Sept. 17, 1936		110.1	11.52
	Oct. 5, 1936		128.6	11.34
	Nov. 5, 1936		138.2	10.92
	May 15, 1937		139.6	10.09
Utah Sweet Spanish	Aug. 30, 1937	0.	78.7	6.87
	Sept. 28, 1937	1.77	96.3	8.50
	Nov. 4, 1937	2.66	96.8	6.91
	Dec. 21, 1937	7.65	113.0	6.72
Yellow Globe Danvers	Sept. 27, 1937	0.	114.2	7.11
	Nov. 3, 1937	.42	125.2	8.16
	Dec. 20, 1937	2.68	151.4	8.25
	Feb. 22, 1938	3.72	144.2	7.83

SUMMARY

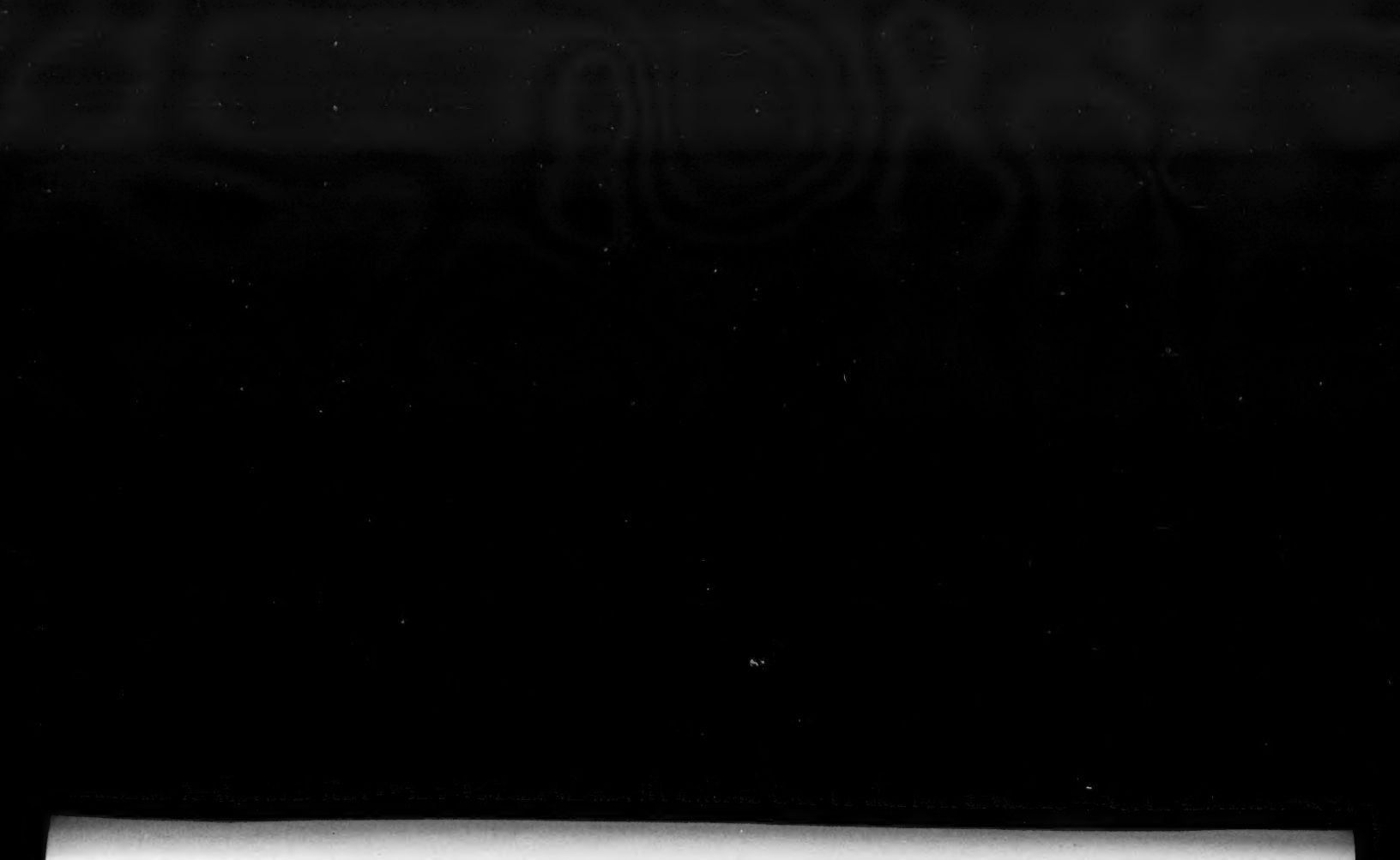
This study is concerned with the pungency of onions as affected by inherited characteristics, growing temperature, soil type, water supply, and storage. The relative pungency of different samples was measured by determining chemically the volatile-sulfur content of the bulbs and thereby their content of onion oil, which is the substance that gives the onion its characteristic pungent taste and odor.

Of the different factors studied, the inherent characteristics of the varieties examined had the most pronounced influence on pungency. Some varieties were found to contain nearly three times as much volatile sulfur as others. On a dry-weight basis, these differences were much smaller, showing that to some extent relative pungency is merely a question of concentration of dry matter.

An experiment in which onions were grown at different temperatures indicates that the pungency of onions tends to increase as the average temperature is raised. However, the results of this experiment must be considered as inconclusive.

Next to variety, the type of soil on which the onions were grown was the most important factor in determining pungency. Onions grown on a peat soil were roughly twice as pungent as the same variety grown on a sandy soil, while onions on loam or a sandy loam were intermediate in pungency. There was a direct correlation between the volatile-sulfur content of the onions and the total sulfur content of the soil on which they were grown. However, this relationship was

not proportional. Where the percentage of sulfur in sandy loam was about 10 times as high as in sandy soil, the corresponding difference in the volatile-sulfur content of the bulbs was only 25 percent.

Overhead irrigation or a high water table always caused a small, but consistent, lowering of the volatile-sulfur content of the bulbs, and this was associated with a pronounced increase in the size of the bulbs grown with an abundant supply of moisture.

In cold storage, over a period of several months onions slowly increased in pungency. This increase may be accounted for in part by the loss of water and carbohydrates as a result of transpiration and respiration.